



CANMET

Compte rendu de
BIOMINET
Proceedings

Edited by Préparé
by par

R.G.L. McCready



SP87-10

88-05503/

**PROCEEDINGS OF THE FOURTH ANNUAL
GENERAL MEETING OF BIOMINET**

NOVEMBER 5, 1987, SUDBURY, CANADA

EDITOR/ÉDITEUR
R.G.L. McCREADY

**COMPTE RENDU DE LA QUATRIÈME RÉUNION
GÉNÉRALE ANNUELLE DE BIOMINET**

5 NOVEMBRE 1987, SUDBURY, CANADA

**CANMET Special Publication
SP87-10**

**Publication spéciale du CANMET
SP87-10**

© Minister of Supply and Services Canada 1988

Available in Canada through

Associated Bookstores
and other bookstores

or by mail from

Canadian Government Publishing Centre
Supply and Services Canada
Ottawa, (Canada) K1A 0S9

Catalogue No. M38-15/87-10E
ISBN 0-660-12866-7

Price subject to change without notice

PROCEEDINGS OF THE FOURTH ANNUAL GENERAL MEETING OF BIOMINET

FOREWORD

BIOMINET presents in this volume the technical papers and one of the poster presentations made by members of BIOMINET at the Fourth annual Meeting which was hosted by Drs. Ferroni and Leduc, Department of Biology, Laurentian University on November 4, 1987.

R.G.L. McCready

Editor

COMPTE RENDU DE LA QUATRIÈME RÉUNION GÉNÉRALE ANNUELLE DE BIOMINET

PRÉFACE

Ce volume comprend les exposés techniques et un des communiqués présentés par les membres de BIOMINET lors de la Quatrième réunion annuelle du 4 novembre 1987 que présidaient M.M. Ferroni et Leduc, du département de biologie de l'Université Laurentienne.

R.G.L. McCready
Éditeur

CONTENTS

SESSION I: BACTERIAL LEACHING

PAPER 1:	UPDATE ON URANIUM LEACHING AT DENISON MINES A. Marchbank.....	3
PAPER 2:	FUNGICIDES FOR CONTROLLING FUNGAL CONTAMINATION DURING LEACHING G. Ferroni.....	19
PAPER 3:	COMPLEX SULPHIDE BASE METAL LEACHING AND ECONOMICS I. Suzuki, H. Lizama, J.K. Oh and P.D. Tackaberry.....	57
PAPER 4:	THE POTENTIAL OF THERMOPHILIC BACTERIA IN THE PRETREATMENT OF REFRACTORY GOLD ORES R.W. Lawrence.....	75
PAPER 5:	BACTERIAL AND CHEMICAL LEACHING OF COMPLEX SULPHIDE ORES V. Sanmugasunderam, W.D. Gould, R.G.L. McCready, S. Rajan, M. Beaulne and P. Mainwaring.....	103

SESSION II: METAL RECOVERY AND ENVIRONMENT

PAPER 6:	OPTIMIZATION OF FUNGAL ADSORPTION OF URANIUM AND CELL IMMOBILIZATION V.I. Lakshmanan, D.J. King, M. Tsezos and R.G.L. McCready.....	119
PAPER 7:	BIORECOVERY OF SELENIUM R.G.L. McCready, J. Salley and T.G. Smith.....	135
PAPER 8:	PROGRESS ON EMULSIFICATION AND FLOTATION AS A MEANS OF UPGRADING A Cu/Mo CONCENTRATE R.G.L. McCready, J. Salley, D.G. Hoppe and D.R. Bartlett.....	147
PAPER 9:	PROGRESS IN THE DEVELOPMENT OF ECOLOGICAL ENGINEERING METHODS FOR CURTAILMENT OF ACID MINE DRAINAGE M. Kalin.....	157

POSTER SESSION

PAPER 10:	DETERMINATION OF ACTIVITY PARAMETERS IN THIOBACILLUS FERROOXIDANS STRAINS AS CRITERIA FOR MINERAL LEACHING EFFICIENCY. I. GROWTH CHARACTERISTICS AND EFFECT OF METALS ON GROWTH AND ON SULFUR OR FERROUS IRON OXIDATION I. Suzuki, J.K. Oh, P.D. Tackaberry and H. Lizama.....	179
-----------	---	-----

SESSION I

BACTERIAL LEACHING



SESSION I: PAPER 1

ABSTRACT

A. Marchbank, Denison Mines Ltd.

In 1984, Denison Mines signed a contract with the federal government under the Program for Industry/Laboratory Projects (PILP). The program allowed Denison Mines to carry out a full-scale production demonstration for uranium recovery with assistance from CANMET laboratories. Stopes were set up to contain an average of 20,000 tons of ore with regular flooding, drainage and aeration to maintain an active bacterial culture on the ore for oxidizing the pyrite and allowing for uranium dissolution. Dr. R.G.L. McCready of Dalhousie and Dr. G.D. Ferroni of Laurentian University investigated temperature and nutrient effects while Dr. P.L. Douglas of Waterloo developed a predictive computer model of a flood leaching stope. Column tests were carried out to test the various process improvements. Repetitive sampling of the ores during the experiments was mandatory in order to obtain representative results of the operating stopes. Ore analysis revealed that the grade of the ore increased in the finest size range (ie. 1/4 in. material was higher than plus 1/4 in. minus 1/2 in.). The tailings grade generally rises with increasing particle size up to 4 - 6 in., then it declines rapidly. The 12 in. grade was found to be of similar grade to the 1/4 in. fraction. Large particles showed a large proportion of barren waste then the small particles due to the breakage in ore along mineralization lines and not through the waste material. The coarse material would give 30% recovery uranium while the fine material is expected to give greater than 90% recovery. 90 flood leaching stopes are presently in operation with over one million tonnes of ore. Over 840,000 lbs of uranium has been recovered from the bacterial leaching.

SESSION I: PRÉSENTATION 1

Résumé

En 1984, la Denison Mines a signé un contrat avec le gouvernement fédéral en conformité avec le Programme des projets industrie/laboratoires (PPIL). Ce programme a permis aux mines Denison d'effectuer en grandeur réelle une démonstration de la production, dans le domaine de la récupération de l'uranium, avec l'assistance des laboratoires du CANMET. On a établi des chantiers d'abattage, contenant en moyenne 20 000 tonnes de minerai, que l'on a soumis à des périodes régulières d'inondation, de drainage et d'aération, de façon à entretenir une culture bactérienne active sur le minerai, qui puisse oxyder la pyrite et permettre la dissolution de l'uranium. M. (Dr) R.G.L. McCready de l'Université Dalhousie et M. (Dr) G.D. Ferroni de l'Université Laurentienne ont étudié les effets de la température et des nutriments, tandis que M. (Dr) P.L. Douglas de l'Université de Waterloo a mis au point un modèle informatique de calcul pour un chantier soumis à la lixiviation par inondation. On a ensuite réalisé des essais en colonne pour vérifier les diverses améliorations du procédé. Il a été obligatoire d'effectuer un échantillonnage répétitif des minerais durant les expériences pour obtenir des résultats représentatifs des chantiers d'exploitation. L'analyse du minerai a révélé que la teneur de celui-ci augmentait dans les dimensions granulométriques les plus fines (par exemple la teneur des produits de 1/4 po était plus élevée que celle des produits entre plus 1/4 po moins 1/2 po). Généralement, la teneur des stériles augmente à mesure que s'élève la dimension granulométrique jusqu'à 4-6 po, puis décline rapidement. On a constaté que la teneur de la fraction de 12 po était similaire à celle de la fraction de 1/4 po. Les particules plus grosses contenaient une plus grande proportion de produits

stériles que les petites particules, en raison de la fracturation granulaire du minerai le long des lignes de minéralisation, et non à travers le produit stérile. Les matériaux grossiers permettent une récupération de 30 % de l'uranium, tandis que les fines devraient permettre une récupération supérieure à 90 %.

Actuellement, 90 chantiers sont soumis à la lixiviation par inondation, et contiennent plus d'un million de tonnes de minerai. On a récupéré au-delà de 840 000 livres d'uranium par lixiviation bactérienne.



UPDATE ON URANIUM LEACHING AT DENISON MINES

Introduction

In early 1982, Denison Mines formed a task force to investigate the feasibility of developing a practical bacterially-assisted leaching method. The company, and several other mines in the Elliot Lake camp, had been using bacterial leaching as a salvage method since the early 1960's. Small amounts of uranium had been produced from waste piles, ore left behind after mining activities and ore-bearing stope pillars. However, no successful attempt had been made to use the technique as a complete mining and production method. Since the ore-body contains large low-grade reserves it was felt that the method could prove to be an economical way of exploiting these reserves.

Initially, the task force concentrated on improving and expanding the salvage leaching operation. Prior to the formation of the task-force, Denison and several other uranium producers had sponsored a study on the mechanisms of the process at the British Columbia Research Council. This contract had greatly increased the knowledge within the company concerning bacterial leaching. The knowledge was put to good use in developing greatly-improved, salvage production methods. Production from these areas increased dramatically during the first year. This provided the group with the credibility and the access to funds necessary to continue development of a full-scale production method. During this period, internal and contract research was initiated to determine the efficiency which could be expected from this method. B.C. Research carried out an additional study for Denison alone to determine the extraction which could be expected from run-of-mine ore using two different leaching methods. Some of the work was duplicated at Denison and extensive field studies were carried out. Laboratory extractions in the neighbourhood of 75% using either a trickle or a modified flood leaching method encouraged the company to proceed with development of the method. The use of trickle leaching to treat

ore specifically broken for the purpose was begun. The Engineering division, with extensive assistance from mining and metallurgical personnel, began the design of a flood leaching system. In early 1984 a contract was signed with the federal government under the Program for Industry/Laboratory Projects (PILP). The agreement provided financial assistance on a cost-sharing basis to allow Denison to carry out a full-scale production demonstration of the technology. The project was administered by Denison with extensive assistance from CANMET Laboratories. The experience of CANMET in this field and the excellent research facilities and personnel contributed a great deal to the success of the project.

The Flood Leaching Method

The extraction method developed at Denison during the project is a modified flood leaching technique. After conventional mining is completed in an area and all of the ore has been removed, the mined-out stopes are prepared for flood leaching. The low-grade ore band lies several feet above the existing stope opening. The stopes are drilled off through their entire length with 2 in. dia. holes on a 3 ft. by 4 ft. grid pattern. The holes are generally about 25 ft. deep. After a small opening has been created in a preliminary blast, the main body of the stope is taken down in a single blast. A single stope can contain up to 50,000 tons of ore, although 20,000 tons would be an average stope. A leaching block generally contains two stopes.

Concrete pressure bulkheads are then constructed to seal off the stopes and allow the ore to be completely submerged in solution. The block is then flooded to wet and inoculate the ore and to test the seal at the bulkheads. The water is immediately drained and any necessary remedial work is completed. The block is then flooded and drained on a regular schedule.

During the rest periods, while the stope is empty, the bacteria become active, oxidizing the pyrite and providing an excellent environment for uranium dissolution. Uranium concentrations in the interstitial moisture rise until a level is reached which is toxic to the bacteria. The block is then flushed with low-grade solution to allow the leach cycle to begin again. Since it is impossible to measure exactly when bacterial activity within the sealed stopes begins to decline, a standard, three-week rest cycle is employed. Flooding and draining the block generally takes approximately one week. Each leaching block, therefore, receives one flush a month and requires eighteen months to reach completion. A target of 70 % extraction in eighteen months was set at the start of the project and still seems realistic.

Flood Leaching Stope Aeration System

To supply the bacteria with the required oxygen and carbon dioxide, aeration lines are installed on the floor of the stope prior to blasting. Initially, these were 0.5 in. dia., perforated, polyethylene hoses fed from the mine compressed air supply. This system provided an adequate quantity of air for bacterial needs. However, with the rapid expansion of flood leaching the use of compressed air has placed excessive strain on the mine compressors. Since the application does not call for high-pressure air, it was decided to design an independent, low-pressure system to do the job.

The system installed is supplied by a Canada Blower and Forge Co. high-pressure fan blower. The fan delivers approximately 200 c.f.m. of air at 30 in.w.g. to each of fifteen stopes in the trial installation. The distribution lines underneath the ore have been increased from 1/2 in. to 2 in. dia. polyethylene hose. This first system has been in operation for

several months now without problems. Preliminary results indicate that a larger volume of air is being fed to the stopes. Also oxidation seems more consistent than with the compressed air system. All future flood leaching blocks will be aerated using this system.

Stope Exhaust System

One of the largest problems arising from the rapid expansion of biological leaching has been the large quantity of broken ore which has accumulated underground. When the ore is broken, there is, naturally, a tremendous increase in the exposed surface area. This causes a significant increase in radon gas emanation rates and in the concentration of airborne radon daughters. The result is that, as the use of leaching and the resultant ore inventory increases, there is mounting pressure placed on the overall mine ventilation system.

To keep ventilation requirements at a minimum, an independent exhaust duct network was installed to service the flood leaching area. Since the leach blocks are all well sealed, the radon gas is held inside the stopes. Aeration is the only feed to the stope during rest periods, displacing a constant volume of air. During this period, however, the increase in the concentration of radon exceeds the rate at which it is displaced. The radiation level in the stope air increases until the level of decay matches the emanation rate, a very high level. The leakage of this air from the stopes quickly contaminates surrounding working areas when several blocks are in operation. In addition, when the blocks are being flooded, all of the stope air is displaced, exaggerating the problem.

To avoid this problem, collection lines are connected to the exhaust holes in each stope. Any remaining drill holes into the stope are sealed. The collector lines draw the contaminated air

into the main duct which transports it directly to the central exhaust corridor. Two large exhaust systems, servicing all of the flood leaching stopes, are in operation at present. The trickle leaching areas, however, cannot be sealed to contain the radiation. Flood ventilation is the only method of maintaining acceptable levels of radon daughters in these areas. This places a strain on the mine ventilation system and is not nearly as effective as the flood leaching system.

University Research Program

Basic research has continued throughout the development program. As part of the PILP project several Canadian universities were contracted to carry out supporting research. In the first phase of the project, Dr. G.D. Ferroni of Laurentian University and Dr. R.G.L. McCready of Dalhousie investigated temperature and nutrient effects. In the second phase Dr Ferroni was again contracted to perform basic research into related fields. Dr P.L. Douglas of Waterloo developed a predictive computer model of a flood leaching stope. Presently, with assistance from Ontario's University Research Initiative Fund, these research programs are continuing. A group at Laurentian, led by Dr Ferroni, is carrying out more research into the effect of temperature on different strains of the bacteria. Dr Douglas is further refining the predictive model. The model will be used as the basis of a program which will help to optimize production scheduling. Denison is closely involved in these research efforts. The knowledge gained through the association with the academic sector has proven invaluable in the development of this process.

In-House Research

While the external basic research has been continuing, on-site research efforts have concentrated on large-scale laboratory testwork and production monitoring. Large-scale column tests were carried out early in the PILP program to test various process improvements. The results from these generally confirmed expectations of leaching efficiency. The present phase of the project is concerned more with providing confirmation of production results.

The normal method of calculating production levels relies on flow monitoring and solution assays. This is generally adequate as far as the day-to-day operation of the circuit is concerned. However, from a metallurgical viewpoint, the method cannot be considered proven unless the solid tailings have been assayed.

The processing of solid tailings for screen analysis and assay is an extremely difficult task. The wide range of sizes within the ore pile means that very large samples must be analyzed to obtain a representative result. The sample size processed at the underground laboratory ranges from 2500 - 3500 lbs. Even this is not really a large enough sample. One 12 in. fragment can weigh over 60 lbs. The average sample, therefore, contains only 3 - 5 pieces in this size range. Since it is not practical to process larger samples, repetitive sampling is necessary to confirm the accuracy of any results.

Adding to the problem is the location of the samples themselves. Ore treated by trickle leaching is simple enough to sample. The areas are accessible to a scoop tram so a series of samples can be easily obtained for analysis. Flood leaching stopes, however, are sealed off from the neighbouring workings by 3 - 5 ft. of reinforced concrete. The inside of the stope is not bolted since, normally, no entry is possible. Finally, the desired sample location is at the top of the ore-pile, 30 ft above the stope opening. The material in the lower half of the

heap comes from the interbedded quartzite layer which contains very little ore. It is, therefore, a very costly proposition to retrieve useful samples from these stopes.

However, as part of the PILP project, a complete evaluation of three different fragmentation patterns was necessary. A complete assessment of the extraction efficiency of the method was also required. The only way to fulfill both of these requirements was to carry out screen analyses of the upper reef portion of the heap. Accordingly, a ramp was driven up from an adjacent stope to intersect the hanging wall of the flood leaching stopes in the project area. Breakthroughs were made in the pillars between the three leaching blocks. The back was bolted across all six stopes to permit safe access for visual observation and tailings sampling.

Sample retrieval and processing from these stopes has been ongoing for some time now. Tables 1 - 5 show the results obtained to date. The two values reported for recovery reflect the range of head grades which could be compared to the tailings assays. Recovery "A" is calculated using the upper reef grade from the diamond drill holes closest to the sample location. The calculation of "B" uses the average stope head grade. As can be seen, the 70 % target has been reached in nearly all cases. In fact, since the upper reef grade should be more accurate than the overall stope grade, the higher results should be correct.

The results do not show conclusively that any one blasting pattern is best. Visual observation of the stopes does suggest that there is some improvement with the finer pattern but several other factors could be responsible for this.

First of all, a scoop tram is not a precision instrument, sampling error could be the reason for the uncertainty. Secondly, the ground conditions in the stopes are variable. Some stopes have very few slabs of rock which have fallen from the

back. Others have a thick layer of waste slabs covering the ore pile. In two of the stopes, the slabs are so large that they make it impossible to take ore from the proper area of the pile. Naturally, if the sample location is not deep enough, some of the back material will be included. Since the slabs are generally quite large, a very few would make a significant difference to the results of a screen analysis.

The analyses do show, however, that all three of the drilling patterns do produce muck which will give acceptable extraction results. Tables 6 and 7 are tailings analyses from a spray leaching stope blasted using a 3 ft. by 4 ft. drilling pattern. If the screen analyses are averaged, the 2 ft. by 4 ft. ore has 73 % passing 4 in. while the 3ft by 4 ft. has 70 % (Not enough 3 ft. by 3 ft. samples have been processed to date to be considered representative). This would not seem to be enough of an improvement to justify a 50 % increase in drilling costs.

The spray leaching stope was operated for several months after it was considered leached-out. It was used to produce acidic solution as a feed for other stopes in the area. This probably explains the excellent extraction results obtained. Recovery "C" in Table 7 represents the extraction calculated using the interbedded quartzite layer average as a head grade. It is probably not feasible to operate flood leaching stopes for such an extended period.

Factors Influencing Final Extraction

If tables 1 - 7 are examined closely, two unusual grade trends can be seen. In nearly all cases, there is an increase in grade in the finest size range. The minus 1/4 in. material is of higher grade than the plus 1/4in. minus 1/2 in. material. Since the samples are processed to reflect actual recovery under a production situation, they are not washed before processing. When the fine material is washed with dilute sulphuric acid, the

grade generally drops to almost half of its previous value. It would seem that some uranium is precipitating out within the heap. This is a relatively minor effect, but it could easily be reducing actual extraction by a percentage point. The mineralogical staff at CANMET is presently studying the tailings samples in an attempt to identify the cause of the problem.

The other grade trend is much more significant in terms of recovery. The tailings grade generally rises with increasing particle size, which is normally expected. However, the rise in grade seems to reach a peak at 4 - 6 in. after which it declines quickly. The 12 in. material generally seems to be of similar grade to the 1/4 in. fraction.

The explanation for this is readily apparent upon visual examination of the particles. The small particles show the normal pebble conglomerate mineralization. The larger particles show a much greater proportion of barren waste which increases with size. Fragments larger than 12 in. usually contain very little mineralization. The fragmentation of the ore is obviously highly preferential. The ore, as should be expected, breaks easily along lines of mineralization. The waste material does not break easily and constitutes the majority of the larger particles. This factor, although it should have been predictable, was not taken into account in previous bench-scale test work. When the Denison project began, many predicted it would fail because of poor extractions in the larger sizes. The fact that there is not a homogeneous break is largely responsible for the success the method has had with such a coarse feed. A completely homogeneous ore distribution would drop recovery by nearly 10 %. Table 8 demonstrates the validity of this point. Very few flood leaching stope head analyses have been performed because of the difficulty of obtaining a representative sample. The only method of retrieving a sample is to draw muck from the stope opening before bulkhead construction. The bottom portion of the ore profile is predominantly interbedded quartzite with a few filaments of ore

passing through it. Naturally the lower portion of the heap would contain mostly this material.

As can be seen, the sample is very coarse, only 44 % passing 4 in. The coarse particles though, have very little uranium contained. A recovery of 30 % from this entire fraction would give a reasonable tail grade. The fine particles are relatively high-grade compared to the stope average. Recovery in excess of 90 % could be expected from these particles.

Conclusion

At present, approximately 90 flood leaching stopes are in varying stages of operation or preparation for leaching. For all leaching methods, an operating inventory of over one million tons of ore is maintained. In 1988 the mine plan calls for 1.1 million tons of low-grade ore to be broken for leaching. This year over 840,000 lbs of uranium will come from bacterial leaching. The use of this method will continue to expand over the coming years. The development of the flood leaching method, while difficult, has been a great success which will have a strong impact on the long-term viability of the Elliot Lake operation.

Acknowledgements

The author would like to thank the management of Denison Mines Ltd for granting permission to publish this report. Also, the management and personnel of Denison, CANMET Laboratories and the three universities have contributed a great deal to the success of this project. The author is very grateful to all parties for their help.

TABLES

Table 1: 40139 Stope Tailings Analysis

Size Fraction	Weight (lbs)	Weight (%)	Grade (lbs/ton)	Recovery (%)
+12 in.	215.3	9.37	0.22	
+8 in.	126.6	5.51	0.31	
+6 in.	133.1	5.80	0.40	
+4 in.	164.7	7.17	0.30	
+2 in.	310.6	13.52	0.21	
+1 in.	317.4	13.82	0.19	
+1/2 in.	312.9	13.62	0.16	
+1/4 in.	213.7	9.52	0.14	
-1/4 in.	497.5	21.66	0.19	A) 78.6
Total	2296.8	100	0.21	B) 72.7

Table 2: 40140 Stope Tailings Analysis #1

Size Fraction	Weight (lbs)	Weight (%)	Grade (lbs/ton)	Recovery (%)
+12 in.	455.8	17.24	0.34	
+8 in.	363.9	13.77	0.46	
+6 in.	230.7	8.73	0.63	
+4 in.	302.8	11.46	0.62	
+2 in.	524.0	19.83	0.33	
+1 in.	229.0	8.66	0.22	
+1/2 in.	156.6	5.92	0.14	
+1/4 in.	189.4	7.17	0.10	
-1/4 in.	190.9	7.22	0.21	A) 69.2
Total	2643.1	100	0.36	B) 60.9

Table 3: 40142 Stope Tailings Analysis #1

Size Fraction	Weight (lbs)	Weight (%)	Grade (lbs/ton)	Recovery (%)
+12 in.	764.5	22.40	0.22	
+8 in.	125.4	3.67	0.38	
+6 in.	136.7	4.00	0.30	
+4 in.	120.0	3.52	0.41	
+2 in.	413.6	12.12	0.31	
+1 in.	404.3	11.84	0.34	
+1/2 in.	413.6	12.12	0.28	
+1/4 in.	324.9	9.52	0.17	
-1/4 in.	710.6	20.82	0.15	A) 77.3
Total	3413.6	100	0.25	B) 70.2

Table 4: 40142 Stope Tailings Analysis #2

Size Fraction	Weight (lbs)	Weight (%)	Grade (lbs/ton)	Recovery (%)
+12 in.	177.3	5.92	0.30	
+8 in.	117.9	3.94	0.70	
+6 in.	81.8	2.73	0.22	
+4 in.	213.9	7.14	0.24	
+2 in.	511.0	17.06	0.31	
+1 in.	453.8	15.15	0.17	
+1/2 in.	419.4	14.00	0.21	
+1/4 in.	307.8	10.27	0.17	
-1/4 in.	713.2	23.8	0.20	A) 78.2
Total	2996.1	100	0.24	B) 71.4

Table 5: 40143 Stope Tailings Analysis #1

Size Fraction	Weight (lbs)	Weight (%)
+12 in.	390.6	16.32
+8 in.	82.4	3.44
+6 in.	105.4	4.40
+4 in.	75.5	3.15
+2 in.	279.3	11.67
+1 in.	371.2	15.51
+1/2 in.	328.9	13.74
+1/4 in.	255.0	10.65
-1/4 in.	505.1	21.1
Total	2393.4	100

Table 6: 46078 Stope Tailings Analysis #1

Size Fraction	Weight (lbs)	Weight (%)	Grade (lbs/ton)	Recovery (%)
+12 in.	261.3	8.03	0.20	
+8 in.	202.1	6.21	0.36	
+6 in.	353.2	10.85	0.40	
+4 in.	136.1	4.18	0.25	
+2 in.	489.7	15.04	0.25	
+1 in.	505.3	15.52	0.22	
+1/2 in.	393.3	12.08	0.09	
+1/4 in.	271.7	8.35	0.09	
-1/4 in.	642.3	19.73	0.17	A) 81.2
Total	3255.0	100	0.22	B) 74.5

Table 7: 46078 Stope Tailings Analysis #2

Size Fraction	Weight (lbs)	Weight (%)	Grade (lbs/ton)	Recovery (%)
+12 in.	471.6	14.2	0.10	
+8 in.	198.2	6.0	0.12	
+6 in.	205.9	6.2	0.21	
+4 in.	260.1	7.9	0.10	
+2 in.	571.6	17.2	0.14	
+1 in.	497.2	15.0	0.14	
+1/2 in.	364.3	10.4	0.10	
+1/4 in.	248.9	7.5	0.09	A) 89.6
-1/4 in.	509.9	15.4	0.13	B) 85.9
Total	3327.7	100	0.13	C) 65.7

Table 8: 50963 Stope Head Analysis

Size Fraction	Weight (lbs)	Weight (%)	Grade (lbs/ton)
+12 in.	542.8	18.26	0.47
+8 in.	427.1	14.37	0.23
+6 in.	287.4	9.67	0.42
+4 in.	399.6	13.44	0.35
+2 in.	647.7	21.79	0.61
+1 in.	328.4	11.05	0.55
+1/2 in.	113.8	3.83	0.32
+1/4 in.	57.6	1.94	0.81
-1/4 in.	168.3	5.66	1.11
Total	2972.7	100	0.49
Predicted UR Grade: 0.98 10: 0.26			

SESSION I: PAPER 2

ABSTRACT

G. Ferroni, Laurentian University

The purpose of the research described in these proceedings was to isolate stope-contaminating fungi from the bioleaching areas of Denison Mines and to test the effectiveness of certain antifungal agents against the isolates.

The yellow-encrusted ore particles which comprised the sample of Sampling I, upon microscopic examination, revealed numerous tiny bacterial cells (presumably Thiobacillus ferrooxidans) but no fungal structures. Two fungal isolates, both Penicillium species, were eventually recovered on fungal isolation media.

Of the twelve fungicides tested, only cycloheximide and amphotericin B had no adverse effect on the growth of Thiobacillus ferrooxidans, and Mertect (45% thiabendazole) had no effect at the two lower concentrations (1 ppm and 10 ppm, thiabendazole) tested. However, the two fungal isolates were resistant to cycloheximide and amphotericin B, whereas they were susceptible to Mertect at a concentration as low as 2 ppm with respect to the active ingredient. The resistance to cycloheximide was evident whether the test medium was a solid or liquid one, whether the pH of the medium was 3.5 or 5.5, and even when the spore suspensions used as inocula were diluted to 10^{-3} . When Mertect at concentrations of active ingredient of 0, 10, 25, 50, 75 and 100 ppm was tested against Thiobacillus ferrooxidans, the results indicated that at some concentration greater than 25 ppm, growth was adversely affected.

For Samplings II and III, thirteen and eight distinct mold types, respectively, were recovered, as well as a few heterotrophic bacteria and yeasts. Nevertheless, none of the samples showed evidence of fungi to the unaided eye or microscopically; and, spore counts obtained from Sampling III were consistent with the fungal population being small.

When Mertect was tested against an additional fifteen fungal isolates, only two showed resistance to thiabendazole at 50 ppm, and four showed some resistance at 10 ppm.

SESSION I: PRÉSENTATION 2

RÉSUMÉ

La recherche décrite dans ces comptes-rendus avait pour objectifs l'isolement des mycètes qui contaminent les chantiers d'abattage à partir des zones des mines Denison soumises à la lixiviation, et la mise à l'épreuve de l'efficacité de certains agents antifongiques vis-à-vis des isolats.

L'examen au microscope a révélé que les particules de minerai incrustées de jaune, qui constituaient l'éprouvette recueillie durant l'échantillonnage 1, contenaient de nombreuses cellules bactériennes minuscules (on suppose qu'il s'agit de Thiobacillus ferrooxidans), mais aucune structure fongique. On a fini par récupérer deux isolats fongiques, tous deux de l'espèce Penicillium, dans des milieux d'isolement des mycètes.

Des douze fongicides mis à l'épreuve, seuls le cycloheximide et l'amphotéricine B n'ont eu aucun effet adverse sur la croissance de Thiobacillus ferrooxidans, et le Mertect (45 % de thiabendazole) n'a eu aucun effet aux deux faibles concentrations expérimentales (1 ppm et 10 ppm de thiabendazole). Toutefois, les deux isolats fongiques se sont avérés résistants au cycloheximide et à l'amphotéricine B, tandis qu'ils étaient sensibles au Mertect à une concentration aussi basse que 2 ppm de l'ingrédient actif. La résistance au cycloheximide a été évidente, que le milieu expérimental soit solide ou liquide, que le pH du milieu soit 3,5 ou 5,5, et cela même lorsque les suspensions de spores servant d'inocula étaient diluées à 10^{-3} . On a fait l'essai du Mertect, aux concentrations d'ingrédient actif de 0, 10, 25, 50, 75 et 100 ppm contre

Thiobacillus ferrooxidans; on a obtenu des résultats indiquant qu'à certaines concentrations dépassant 25 ppm, se manifestait un effet négatif sur la croissance.

Dans le cas des échantillonnages II et III, on a récupéré respectivement treize et huit types de moisissures, ainsi que quelques bactéries et levures hétérotrophes. Néanmoins, l'examen à l'oeil nu ou au microscope n'a indiqué dans aucun des échantillons la présence de mycètes; et les résultats de la numération des spores provenant de l'échantillon III concordaient avec la faible population fongique.

Lorsqu'on a de nouveau fait l'essai du Mertect sur quinze isolats fongiques supplémentaires, seuls deux de ces derniers ont montré une résistance au thiabendazole présent à la concentration de 50 ppm, et quatre ont montré un certain degré de résistance à ce produit, à la concentration de 10 ppm.

FUNGICIDES FOR CONTROLLING FUNGAL CONTAMINATION DURING LEACHING

INTRODUCTION

In the bioleaching of uranium from uranium-containing ores, insoluble tetravalent uranium oxide (UO_2) is converted to the leachable hexavalent species (UO_2SO_4) by ferric ions (Fe^{3+}), according to the following equation:



Fe^{3+} is the oxidant in this reaction and it is generated by the chemolithotrophic bacterium Thiobacillus ferrooxidans which oxidizes the ferrous (Fe^{2+}) iron accompanying uranium ores, according to the equation:



Therefore, the rate of bioleaching of uranium is directly related to the rate at which the bacterium oxidize Fe^{2+} to provide energy for its growth requirements; the factors such as temperature (Ferroni, Leduc and Todd; 1986), acidity, Fe^{2+} concentration, oxygen concentration, carbon dioxide concentration, and nutrient availability, which influence the growth rate of Thiobacillus ferrooxidans, determine the efficiency of the bioleaching process.

A potential problem in some of the Denison Mines leaching areas is the existence of fungi which apparently adsorb solubilized uranium and, possibly, interfere with the contact between Thiobacillus ferrooxidans and its substrate. Work done by R.G.L. McCready of the Canada Centre for Mineral & Energy Technology (unpublished data), prior to the commencement of this

study, resulted in the recovery of an acidophilic Cryptococcus species, a Penicillium species, a Rhizopus - like species, and at least one bacterium which is an actinomycete or streptomycete. These heterotrophs are acidophilic and uranium tolerant, and presumably use hydrocarbons, which are present in the oils used underground, as a carbon source.

The objectives of the research being described were to isolate the stope-contaminating fungi from the bioleaching areas of Denison Mines and to test the effectiveness of some antifungal agents against these isolates.

MATERIALS AND METHODS

Growth media

Initial fungal isolations employed the following growth media: potato dextrose agar (PDA); Sabouraud dextrose agar (SDA); YM agar (YMA); Bacto-yeast malt extract agar (BYMEA); and nutrient agar (NA). Each medium was prepared at two different pH's, a non-adjusted pH (usually pH 6 to 6.5), and a pH of less than 4.0. For the non-adjusted pH, the pH of the medium was that following rehydration of the commercial product and autoclaving. For the adjusted pH, an appropriate amount of sterile 10% tartaric acid was added after autoclaving, and it was necessary to increase the amount of agar from 1.5% to 3.0 - 3.5% to ensure that appropriate solidification occurred.

The medium employed for the growth of Thiobacillus ferrooxidans was the modified 9K medium of Silverman and Lundgren (1959), herein referred to as medium 9K. The final pH was usually in the range between 2.6 and 2.8 and the Fe^{2+} concentration was around 8,800 ppm.

Sample description and processing for Sampling I

The first sample collected was stope muck. The muck was encrusted with a yellow material.

One to two g of the yellow crust were scraped off and suspended in 3 to 4 mL of sterile mineral salts solution contained in a Petri dish. This suspension was examined using phase contrast microscopy, and light microscopy after methylene blue staining. The pH was determined using a pH meter and 1 mL of the suspension was added to 10 mL of Beckman HP/b Ready-Solv cocktail for liquid scintillation counting according to the method of Leduc, Ferroni and Belcourt (1987), in order to determine its extent of radioactivity.

Fifteen g of yellow crust from the pebbles were added to 135 mL of sterile saline (0.8%) in a 225-mL Erlenmeyer flask. The flask was shaken at high speed for 2 min. Next, 0.1-mL aliquots were added to duplicate plates of PDA, SDA, YMA, BYMEA, and NA, at the two pH's. The inoculum was spread with sterile, bent glass rods. One set of plates was incubated at 20°C and the other set was incubated at 37°C. Also, 5 mL of the suspension were added to 50 mL of medium 9K, and incubated at 20°C with shaking for the recovery of Thiobacillus ferrooxidans.

The effect of antifungal agents on the growth of Thiobacillus ferrooxidans

A list of the antifungal agents employed, along with their descriptions, is found in Table 1.

The growth of Thiobacillus ferrooxidans in medium 9K, supplemented with the antifungal agent of interest, was followed by the colorimetric measurement of Fe^{3+} (Kolthoff et al., 1969) produced as a result of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ oxidation by the bacterium. The inoculum size was always 3 mL in 40 to 47 mL of medium contained in a 250-mL Erlenmeyer flask, with water being used to bring the total volume to 50 mL, when necessary. Incubation was at 20, 25 or 28°C with shaking at an intermediate speed setting. As culture dilutions of between 1:500 and 1:2,000 were required, 25 to 100 μL were sampled using a Gilson Pipetman (P-100), and transferred to a 50-mL volumetric flask. Next, 25.0 mL of standardized KSCN solution were pipetted into the volumetric and 0.2 M HNO_3 was added up to the mark. After the vessel was thoroughly shaken, the absorbance of the solution was measured with a LKB Novaspec Spectrophotometer at a wavelength of 480 nm. Actual Fe^{3+} values were determined from a standard curve represented by the equation

$$y = 0.191x + 0.0178,$$

where y is the measured absorbance and x is the $[\text{Fe}^{3+}]$ in ppm.

To calculate generation times (g), growth curves in the form of $\ln Fe^{3+}$ as a function of incubation time (h) were constructed. Since the slope of the line representing the logarithmic growth phase is equal to the growth rate constant u, g can be calculated from the relationship $g = 0.693/u$.

(i) Botran, Thiram, Ferbam and PCP#23052. A stock 25,000 ppm solution/suspension (12.5 g in 500 mL) of each fungicide was prepared. The prepared suspensions varied from a thick liquid to a slurry which required constant mixing when being sampled. The concentrations tested were 2.5, 10, and 100 ppm.

(ii) Cycloheximide. A stock solution of 500 ppm was prepared, and additions were made to the experimental flasks to give final concentrations of 1, 10 and 100 ppm.

(iii) Bravo 500, Mertect and Benlate. For these three antifungal agents, stock 1000 ppm solutions/suspensions were prepared based on the active-ingredient percentages supplied by the manufacturer. Bravo 500 and Mertect formed solutions, whereas Benlate formed an insoluble slurry. The concentrations tested were 1, 10 and 100 ppm.

(iv) PCP#53010 and PCP#7644. Stock solutions of 1000 ppm were prepared and concentrations of 1, 10 and 100 ppm were used.

(v) Sapstain and Amphotericin B. Stock 1000 ppm solutions were prepared and appropriate volumes were added to inoculated culture media to give final fungicide concentrations of 1, 10 and 100 ppm. The amphotericin B at 1000 ppm was not completely solubilized, a definite precipitate forming upon standing.

Preparation of fungal spore suspensions

Each fungus was purified by repeated transfers on SDA. For the preparation of spore suspensions, 10 mL of sterile, distilled H_2O were added to a plate containing abundant fungal mass,

followed by the addition of 1 drop of filter-sterilized Tween 80. The plate was placed on a shaker for 2 min. to free as many spores as possible. The spore suspension was removed from the plate by means of a sterile pasteur pipet. After vortexing the suspension for 30 min., its optical density was determined at 650 nm. The spore suspensions were refrigerated if they were not to be used immediately.

The effect of cycloheximide on the growth of fungal isolates D-1-1 and D-1-2

Batches (250 mL) of SDA were prepared and appropriate volumes of a filter-sterilized 500 ppm solution of cycloheximide were aseptically added to cooled molten medium to give final cycloheximide concentrations of 1, 10, 100, 200, 400 and 800 ppm. The pH of the medium was 5.7. The optical densities at 650 nm for the spore suspensions of D-1-1 and D-1-2 were 0.058 and 0.064, respectively. The inoculum size was 0.05 mL which was spread using a bent glass rod and a Fisher Rotoplate. The plates were incubated at 20°C.

The effect of amphotericin B on the growth of fungal isolates D-1-1 and D-1-2

A 1000 ppm stock solution of amphotericin B was used to prepare SDA plates (pH 5.7) having amphotericin B concentrations of 1, 10 and 100 ppm. Because the amphotericin B had a limited solubility in the agar medium, the higher concentrations (200, 400 and 800 ppm) were prepared by adding appropriate weights of the antibiotic powder directly to the molten medium, and the pH was reduced to 3.0 by the addition of sterile, 10% tartaric acid. Nevertheless, tiny specs of the yellow powder were still visible in the solidified plates. The inoculation procedure and conditions of incubation were as already described.

The effect of cycloheximide on the growth of broth cultures of D-1-1 and D-1-2

Sabouraud dextrose broth (neopeptone, 10 g; dextrose, 20 g; distilled H₂O, 1 L; pH 5.6) was prepared and 10 mL volumes were added to culture tubes. Appropriate volumes of the stock 1000 ppm solution of cycloheximide were added to give final concentrations of 1, 10 and 100 ppm. The inoculum size for both fungal spore suspensions was 0.05 mL, and incubation was at 20°C under stationary conditions.

The effect of spore concentration on the effectiveness of cycloheximide

SDA plates containing 100 ppm of cycloheximide were prepared at two pH 5.5 and 3.5. The isolates D-1-1 and D-12 were grown on SDA, without the antibiotic, and spore suspensions were prepared as already described. Dilutions of 10^{-1} to 10^{-3} were made from the original spore suspension (10°C), and spore counts were determined for each isolate by inoculating 0.1 mL of the 10^{-1} and 10^{-3} dilutions onto triplicate, pH 5.5, SDA plates. Spore dilutions (10^0 to 10^{-3}), for each isolate, were inoculated (0.1 mL) on to SDA plates (pH 5.5 and 3.5) containing cycloheximide, in the usual manner. Incubation was at 20°C.

The effect of Mertect on the growth of D-1-1 and D-1-2

SDA plates containing final concentrations of 1, 10 and 100 ppm of thiabendazole, the active ingredient in the Mertect preparation, were prepared at pH 3.9. This was done by adding appropriate volumes of a filter-sterilized, 1000 ppm solution to the cooled, molten agar medium and adjusting the pH with tartaric acid. The preparation of spore suspensions of D-1-1 and D-1-2, the method of plate inoculation, and the conditions of incubation were as previously described.

Additionally, an experiment was run for which the final concentrations of thiabendazole were 1, 2, 4 and 8 ppm. The test fungi were isolates D-1-1 and D-1-2, and the pH of the SDA medium was 5.7.

The effect of Mertect on the growth of Thiobacillus ferrooxidans

Appropriate volumes of the 1000 ppm stock preparation (based on the active ingredient thiabendazole) were added to medium 9K contained in 250-mL Erlenmeyer flasks, to give final thiabendazole concentrations of 10, 25, 50, 75 and 100 ppm. Inoculation with 3 mL of actively growing Thiobacillus ferrooxidans brought the total volume in a reaction vessel to 50 mL. Duplicate vessels were incubated at 35°C in a reciprocating shaker, and Fe³⁺ determinations were done periodically.

Sample description and processing for Sampling II

Sample D-2A was collected from the floor of an older leaching stope, and consisted of yellow-encrusted pebbles of uranium ore. The crust was easily scraped off or washed off. Sample D-2B was a slime-like material collected from the face of a drift in a main passageway. A steady stream of water from an overflowing sump was flowing down the face of the drift at the sampling point. The slime material hung in long filaments (15 to 20 cm) in the water stream and adjacent to it. This brownish-yellow slimy material was collected using a sterile spatula.

Microscopic examinations were by means of phase contrast microscopy. Five g of each sample (scrapings in the case of D-2A) were added to 250-mL Erlenmeyer flasks containing 45 mL of sterile, distilled water. The flasks were shaken at high speed for 5 min. The pH of subsamples was determined and 1-mL amounts were removed for determination of radioactivity using liquid scintillation counting. Finally, 3 mL of each suspension were added to 50 mL of medium 9K for the isolation of Thiobacillus ferrooxidans, and the suspensions were used to seed the various plates containing fungal growth media. The plates were incubated at 20°C. Fungal isolates were repeatedly transferred to fresh media until pure cultures had been obtained. Isolates were differentiated according to gross morphology and, if necessary, microscopic morphology.

Sample description and processing for Sampling III

Samples D-3A, D-3B, D-3C and D-3D were liquid samples collected from the outfall sumps in stopes 30201, 38034, 32881, and 48047, respectively. Samples D-3X, D-3Y and D-3Z were muck samples collected from the floors of stopes 30201, 38034, and 46078, respectively.

One-hundred mL aliquots of D-3A and D-3B and 50-mL aliquots of D-3C and D-3D were filtered through 0.45-um membrane filters. The filters were transferred to 250-mL Erlenmeyer flasks containing 50 mL of sterile, distilled water. Five g (wet weight) of each solid sample were transferred to flasks containing 45 mL of water. All flasks were shaken at intermediate speed for 15 min. Dilutions of 10^{-1} and 10^{-2} were made for the liquid samples and of 10^{-1} to 10^{-3} for the solid samples. Plating of 0.1 mL was done in triplicate onto pH 3.5 SDA medium and onto pH 5.5 SDA medium, using the spread-plate technique. For the liquid samples only, undiluted samples were plated as well. Incubation was at 20°C, after which the plates were counted for colonies.

The overall effect of Mertect

The preparation of SDA plates containing appropriate concentrations of Mertect, based on its active ingredient thiabendazole, and the experimental design were as previously described. Spore suspensions of the following fungal isolates were tested: D-2A-1; D-2A-2; D-2A-3; D-2A-5; D-2A-8; D-2A-9; D-2B-1; D-2B-2; D-2B-3; D-2B-4; D-2B-7; D-3A-2; D-3Z-1; D-3Z-3; and D-3Z-4.

RESULTS

Sample processing for Sampling I

The suspension of yellow crusted material, upon microscopic examination, showed numerous bacilli. Phase contrast microscopy and light microscopy of methylene blue-stained smears did not reveal any fungal structures.

The pH of the scrapings (5 g of crusted material in 10 mL of H₂O) was 3.4, and 1 mL of sample gave a liquid scintillation count, corrected for background, of 663 disintegrations per minute (dpm).

Fungal growth was luxuriant at the lower incubation temperature for both low and high pH media, but little or no growth occurred at the higher incubation temperature. Most abundant growth occurred on SDA. The two colony types which were recovered were subcultured on SDA a number of times and stock cultures were established. Type 1 (D-1-1) was white and powdery with a central blue-green area. Conidiophores were present and the hyphae were nonseptate. Type 2 (D-1-2) was white and cottony, and no sporangia or conidia were observed. The hyphae were also nonseptate. These types were later identified as different Penicillium species.

Within one week of inoculation, a reddening of the 9K medium indicated the recovery of Thiobacillus ferrooxidans from

the stope muck sample. This culture was subcultured in medium 9K and a stock culture was established.

The effect of antifungal agents on the growth of Thiobacillus ferrooxidans

Table 2 shows the effects of the various antifungal agents on the growth rate of Thiobacillus ferrooxidans, and Table 3 the effects on the extent of iron oxidation by Thiobacillus ferrooxidans. As expected, the antibiotics cycloheximide and amphotericin B neither affected the growth rate (Table 2) nor the amount of Fe^{3+} generated (Table 3) by the bacterium. The antifungal agent Mertect had an adverse effect at the highest concentration tested (100 ppm) only, and the antifungal PCP#53010 affected growth rate and Fe^{3+} production at both 10 ppm and 100 ppm. All the other chemicals affected growth rate and Fe^{3+} production at all concentrations tested. The growth rate differences exhibited by the control cultures were mainly due to different temperatures of incubation.

The effect of cycloheximide on the growth of D-1-1 and D-1-2

Preliminary experiments had indicated that the two isolates were resistant to cycloheximide in the concentration range 1 to 10 ppm, whether the pH of the test SDA medium was 3.7 or 5.7. Table 4 shows the effect of a broader range of cycloheximide concentrations on the growth of D-1-1 and D-1-2. Both isolates exhibited a high degree of resistance. For both isolates, some

growth even occurred at 800 ppm, the highest concentration tested.

The effect of amphotericin B on the growth of D-1-1 and D-1-2

The results for amphotericin B are summarized in Table 5. Fungal isolate D-1-1 showed a high degree of resistance to the antibiotic, whereas fungal isolate D-1-2 showed no growth at concentrations of 200, 400 and 800 ppm.

The effect of cycloheximide on the growth of broth cultures of D-1-1 and D-1-2

Resistance to cycloheximide was also evident when the test medium was a broth as opposed to a solid medium (Table 6). Generally, growth was poorer in the broth, but there was some resistance to all three concentrations tested, 1, 10 and 100 ppm.

The effect of spore concentration on the effectiveness of cycloheximide

The effect of spore concentration in determining the resistance of D-1-1 and D-1-2 to cycloheximide is found in Table 7. For both fungal isolates, as would be expected, the greater the number of spores inoculated onto a plate the greater the apparent resistance to the antibiotic. Nevertheless, when 10^{-3} dilutions of the spore suspensions were used as sources of inoculum, resistance was still evident. The pH, 3.5 or 5.5, of the test medium was not a factor in the pattern of resistance.

The greater resistance of D-1-2 in this particular experiment was due its spore suspension having a higher titre, 17,000 per mL as opposed to 1,400 per mL for D-1-1.

The effect of Mertect on the growth of D-1-1 and D-1-2

Table 8 summarizes the effect of the fungicide Mertect on the growth of the fungal isolates recovered from the first sampling. The concentrations 1, 10 and 100 ppm were, in fact, concentrations of the active ingredient of Mertect, thiabendazole. No growth of D-1-1 or D-1-2 occurred at 10 ppm and 100 ppm. At 1 ppm, growth was slightly reduced for isolate D-1-1 and moderately reduced for isolate D-1-2.

The results for the concentration range 0 to 8 ppm are shown in Table 9. No growth of either D-1-1 or D-1-2 occurred at 2, 4 and 8 ppm thiabendazole, indicating that concentrations greater than 1 ppm are appropriate for the control, of these fungi at least.

The effect of Mertect on the growth of Thiobacillus ferrooxidans

The effect of Mertect on the growth of Thiobacillus ferrooxidans, as measured by the production of Fe^{3+} in cultures, is shown in Table 10. Previous experiments had indicated that growth was prevented at 100 ppm thiabendazole but not at 10 ppm thiabendazole. The inhibitory concentration of thiabendazole for Thiobacillus ferrooxidans was in the range 25 to 50 ppm. No growth occurred at 75 and 100 ppm; at 50 ppm, the growth rate was

extremely slow, there being a maximum Fe^{3+} production of 822 ppm at 71.5 h when the experiment was terminated.

Sample processing for Sampling II

Neither sample D-2A nor sample D-2B showed visual evidence of fungal contamination. Microscopic examination did not reveal any fungal structures, but rather the presence of numerous tiny bacilli, presumably Thiobacillus ferrooxidans.

Sample D-2A (muck scrapings) had a pH of 4.3 and sample D-2B a pH of 3.0. The counts of radioactivity were 858 and 171 dpm for samples D-2A and D2B, respectively.

A description of the heterotrophic microbial isolates is given in Table 11. Thirteen of the isolates were molds, one was a yeast, and two were bacteria.

Sample processing for Sampling III

The results of enumerating four different liquid samples and three muck samples for fungal spores are presented in Table 12. Only three of the seven samples yielded fungal colonies, and even in these samples the counts were not high. A total of eight different fungal types were recovered from these plates, and none was a Penicillium or Penicillium-like fungus.

The sensitivity of fungal isolates to Mertect

Table 13 shows the effect of Mertect on the growth of a number of fungi recovered from Samplings II and III. Nine of the fifteen isolates were completely inhibited from growing at 10 ppm

thiabendazole, four were inhibited at 50 ppm, and only two were resistant to the highest concentration (50 ppm) of thiabendazole tested.

DISCUSSION

The twelve fungicides which were collected were first tested to determine if they adversely affected the growth of Thiobacillus ferrooxidans. Obviously, any chemical which kills or inhibits the growth of the bacterium is not a candidate for use in the mine, as the bioleaching process itself would be adversely affected. The results of Tables 2 and 3 indicated that cycloheximide and amphotericin B were candidates for the control of stope-contaminating fungi, as was Mertect at concentrations below some concentration between 10 and 100 ppm. Those antifungals which had an affect on Thiobacillus ferrooxidans diminished the growth rate, as indicated by generation times being calculable, and restricted amount of growth, as indicated by the depressed maximum ferric iron readings.

Tests of these fungicides against the two Penicillium species recovered from the initial sampling of the mine environment revealed that both isolates possessed a high degree of resistance to cycloheximide (Table 4); and that one of the isolates possessed a high degree of resistance to amphotericin B, with the other isolate being susceptible only to the higher (200, 400 and 800 ppm) concentrations tested (Table 5). These results were somewhat surprising as cycloheximide and amphotericin B are antibiotics supposedly effective against eucaryotic cells. Cycloheximide is an inhibitor of protein synthesis in eucaryotic cells and amphotericin alters cell membrane permeability in eucaryotic cells.

To positively eliminate cycloheximide as a candidate, additional experiments were conducted. The resistance observed when cycloheximide was incorporated into the solid growth medium was also evident when it was into the liquid growth medium (Table 6), which eliminated the possibility that the observed resistance was due to the antibiotic being 'tied up' by some complex involving the agar. Even when fungal spore concentration in the inoculum was diluted to 10^{-3} , both isolates showed a degree of

resistance to 100 ppm of cycloheximide (Table 7), thus eliminating the possibility that the preferred lower test pH was inactivating the antifungal.

When Mertect was tested against the two fungi, it proved to be effective at 10 ppm, that is ten parts of active ingredient thiabendazole per million (Table 8). A subsequent experiment showed an even greater effectiveness for the Mertect, as fungal growth was prevented at a concentration as low as 2 ppm (Table 9). Therefore, the critical concentration of thiabendazole in Mertect with respect to the sensitivity of Thiobacillus ferrooxidans was examined (Table 10). The results warranted the conclusion that concentrations of thiabendazole greater than 25 ppm had an adverse effect on the growth of the uranium-leaching bacterium Thiobacillus ferrooxidans. In other words, low concentrations of the antifungal agent could be used in the mine environment without fear of retarding the bacterium-mediated leaching process.

Using colonial morphology and microscopic morphology as differentiators, two, thirteen and eight distinct mold types were recovered from Samplings I, II and III respectively. Also recovered were a few heterotrophic bacteria and yeasts. In none on the mine samples obtained, however, were fungi visible to the unaided eye, and microscopic examinations even failed to reveal any fungal structures. Also, the spore counts conducted for Sampling III were consistent with the fungal population being very small (Table 12). The highest count for a liquid sample (Stope 46078) 5,667 per g. In fact, for four of the seven samples, the spore count was lower than could be detected by the procedure being used. These results are consistent with a conclusion that during the course of this study fungi were not a nuisance with respect to bacterial leaching process.

In total, Mertect was tested against seventeen fungal isolates (Tables 8 and 13). Of these, only two showed resistance to thiabendazole at 50 ppm, and four showed some resistance to thiobendazole at 10 ppm. The rest were unable to grow at concentrations of either 1 or 10 ppm. Mertect, therefore, is

toxic to a variety of fungi recoverable from the stopes of Denison mines, and especially against the Penicillium species which were by far the numerically-predominant fungal isolates. The active ingredient thiabendazole is toxic due to its conversion to a nitrogenous base analogue. This substance inhibits DNA synthesis or some closely-related aspect of cellular or nuclear division in fungi (Clemons & Sisler; 1971).

MAJOR CONCLUSIONS

The lack of visible evidence of fungal growth in the sampled areas and the absence of microscopically-detectable fungal structures in the samples collected, suggest that during the course of this investigation stope-contaminating fungi were not a problem in the bioleaching areas. The fact that fungi were usually recoverable from samples is probably a result of the constant dissemination of fungal spores by the mine ventilation system.

Should it become necessary to use a fungicide to control fungal growth in a bioleaching area, the fungicide must be chosen judiciously, as most of the antifungals tested in this study were not selectively toxic. That is, they also prevented the growth of or considerably slowed the growth of the leaching bacterium Thiobacillus ferrooxidans.

The best of the antifungals tested was Mertect, the active ingredient being 45% thiabendazole. Most of the fungal isolates tested against Mertect showed susceptibility, and at concentrations which did not effect Thiobacillus ferrooxidans. Therefore, a thiabendazole-based fungicide or pure thiabendazole is a candidate for the control of stope-contaminating fungi in bioleaching areas of the mine, but the use of such should be preceded by routine testing of the degree of resistance of Thiobacillus ferrooxidans to the preparation in question.

ACKNOWLEDGEMENTS

I am indebted to Mr. Marty Todd for his skillfull technical assistance and diligence. I wish to thank Mr. Andrew Marchbank of Denison Mines and Dr. R.G.L. McCready of the Canada Centre for Mineral & Energy Technology for their cooperation and expert advice.

BIBLIOGRAPHY

Clemons, G.P. and H.D. Sesler. (1971). Pestic. Biochem. Physiol. 1: 32-43.

Ferroni, G.D., L.G. Leduc and M. Todd. (1986). Isolation and temperature characterization of psychrotrophic strains of Thiobacillus ferrooxidans from the environment of a uranium mine. J. Gen. Microbiol. 32: 169-175.

Kolthoff, I.M., E.B. Sandell, E.J. Meehan and S. Bruckenstein. (1969). In Quantitative Chemical Analysis. Macmillan, London, 4th edition. pp 1049-1052.

Leduc, L.G., G.D. Ferroni and D. Belcourt. (1987). Liquid scintillation counting as a means of measuring uranium leached from low-grade ores by Thiobacillus ferrooxidans. Hydrometallurgy 18: 255-263.

Silverman, M.P. & D.G. Lundgren. (1959). Studies on the chemoautotrophic bacterium Ferrobacillus ferrooxidans. J. Bacteriol. 77: 642-647.

TABLES

Table 1. The antifungal agents

Chemical	Source	Description
Botran	C	2,6-dichloro-4-nitroaniline; bright yellow powder; slightly soluble in water
Thiram	C	tetramethylthiuran; white powder; slightly soluble in water
Ferbam	C	a metallic dithiocarbamate; black powder; slightly soluble in water
PCP#23052	A	bis-trichloromethylsulfone; thick honey-coloured liquid; soluble in water
Cycloheximide	S	antibiotic isolated from the bacterium <u>Streptomyces griseus</u> ; soluble in water
Bravo 500	C	40.4% chlorothalonil; pale yellow liquid; soluble in water
Mertect	C	45% thiabendazole; thick, yellow liquid; soluble in water
Benlate	C	50% benomyl; white powder; insoluble in water
PCP#53010	A	dark brown liquid; soluble in water
PCP#7644	A	dark brown liquid; soluble in water
Sapstain	A	water soluble fungicide
Amphotericin B	S	polyene antibiotic produced by a <u>Streptomyces</u> species; soluble in water

Codes: C - Chipman Chemicals
 A - Alchem Limited
 S - Sigma Chemical Company

Table 2. The effect of fungicides on the growth of T. ferrooxidans: A summary of generation times

Chemical	Temp (°F)	Generation Time (h)			
		Fungicide Concentration			
		0 ppm	2.5 ppm	10 ppm	100 ppm
Botran	25	27	133	173	209
Thiram	25	27	169	192	196
Ferbam	25	27	187	165	194
PCP#23052	25	27	223	169	216
		Fungicide Concentration			
		0 ppm	1 ppm	10 ppm	100 ppm
Cycloheximide	28	19	19	19	19
Bravo 500	28	19	71	104	67
Mertect	28	19	19	19	85
Benlate	28	19	83	184	98
PCP#53010	28	19	14	56	159
PCP#7644	28	19	63	100	96
Sapstain	25	17	25	131	97
Amphotericin B	20	19	19	24	19

Table 3. The effect of fungicides on the growth of T. ferrooxidans: A summary of maximum ferric iron readings

Chemical	Temp (°C)	Maximum Ferric Iron (ppm)			
		Fungicide Concentration			
		0 ppm	2.5 ppm	10 ppm	100 ppm
Botran	25	6125	1359	1066	891
Thiram	25	6125	1116	980	938
Ferbam	25	6125	1051	1119	967
PCP#23052	25	6125	854	816	769
		0 ppm	1 ppm	10 ppm	100 ppm
Cycloheximide	28	5164	5530	5592	5279
Bravo	28	5060	886	698	761
Mertect	28	5060	5258	5164	591
Benlate	28	5060	860	527	596
PCP#53010	28	5300	5530	1017	727
PCP#7644	28	5300	1044	766	706
Sapstain	25	5478	2594	557	612
Amphotericin B	20	6146	6073	6219	5676

Table 4. The effect of cycloheximide on the growth of fungal isolates D-1-1 and D-1-2

Isolate	Relative Amount of Growth						
	Cycloheximide Conc. (ppm)						
	1	10	100	200	400	800	Control
D-1-1	++++	+++	++	++	++	++	+++++
D-1-2	++++	++++	++	++	++	+	+++++

+ 's indicate the relative amount of growth on plates, with the amount on a control plate being the maximum

Table 5. The effect of amphotericin B on the growth of fungal isolates D-1-1 and D-1-2

Isolate	Relative Amount of Growth						
	Amphotericin B Conc. (ppm)						
	1	10	100	200	400	800	Control
D-1-1	++++	+++	+++	+++	+++	+++	+++++
D-1-2	++++	+++	++	-	-	-	+++++

+ 's indicate the relative amount of growth on plates, with the amount on a control plate being the maximum

Table 6. The effect of cycloheximide on the growth of broth cultures of fungal isolates D-1-1 and D-1-2

Isolate	Relative Amount of Growth			
	Cycloheximide Conc. (ppm)			
	1	10	100	Control
D-1-1	++++	++	+	++++
D-1-2	+++	++	+	++++

+ 's indicate the relative amount of growth in broth tubes, with the amount in a control tube being the maximum

Table 7. The effect of spore concentration of D-1-1 and D-1-2 on resistance to 100 ppm of cycloheximide

		Relative Amount of Growth							
		pH 3.5				pH 5.5			
Isolate	Dilution	Replicate				Replicate			
		1	2	3	C	1	2	3	C
D-1-1	1:10	+++	+++	+++	+++++	+++	+++	+++	+++++
	1:100	++	++	++	+++++	++	++	++	+++++
	1:1000	+	+	+	+++++	+	+	+	+++++
D-1-2	1:10	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
	1:100	++++	++++	++++	+++++	+++	+++	+++	+++++
	1:1000	+++	+++	+++	+++++	++	++	++	+++++

+'s indicate the amount of growth on plates relative to the control plates (C) which contained no cycloheximide

Table 8. The effect of Mertect on the growth of fungal isolates D-1-1 and D-1-2

Isolate	Relative Amount of Growth			
	Active ingredient conc. (ppm)			
	1	10	100	Control
D-1-1	++++	-	-	+++++
D-1-2	+++	-	-	+++++

+'s indicate the relative amount of growth on plates, with the amount on a control plate being the maximum

A - the active ingredient is 45% thiabendazole.

Table 9. The effect of low concentrations of Mertect on the growth of fungal isolates D-1-1 and D-1-2

Isolate	Relative Amount of Growth				
	Active Ingredient Conc. (ppm)'\$				
	1	2	4	8	Control
D-1-1	+++	-	-	-	++++
D-1-2	+++	-	-	-	++++

+'s indicate the relative amount of growth on plates, with the amount on a control plate being the maximum

A - the active ingredient is 45% thiabendazole

Table 10. The effect of Mertect on the growth of
T. ferrooxidans

Incubation Time (h)	Ferric Iron (ppm)'\$					
	Thiabendazole Concentration (ppm)					
	0	10	25	50	75	100
0	330	454	396	328	369	330
23	1268	992	939	622	472	460
29.5	1906	1451	1247	513	424	419
47.5	5079	3699	3189	681	358	297
53.5	4921	4451	3976	776	333	345
71.5	4428	4251	4528	822	211	234

A - average of two replicates and corrected for the amount of
chemical oxidation of Fe.

Table 11. Description of the microbial isolates from Sampling II

Isolate	Description
D-2A-1	Dense, cottony, reddish-orange, fast-growing fungus.
D-2A-2	Diffuse, cottony, light-coloured fungus.
D-2A-3	Dense, powdery, white, wrinkled, fast-growing fungus; the most numerous isolate.
D-2A-4	Powdery, white, greenish-centered, <u>Penicillium</u> -like fungus.
D-2A-5	Dense, small, white, yellowish-green centered, slow-growing fungus.
D-2A-6	Pearly white, mucoid, bacterium.
D-2A-7	Shiny white, bacterium.
D-2A-8	Dense, creamy white, slow-growing fungus.
D-2A-9	Dense, creamy white, fungus with needle-like projections.
D-2B-1	Small, cottony, white to grey, fungus.
D-2B-2	Dense, small, coal black to greyish, slow-growing fungus.
D-2B-3	Dense, cream coloured, slow-growing fungus.
D-2B-4	Orange, shiny, yeast.
D-2B-5	Dense, small, black, slow-growing fungus.
D-2B-6	Dense, creamy white, wrinkled fungus with needle-like projections.
D-2B-7	Powdery, white, large, <u>Penicillium</u> -like fungus.

Table 12. Enumeration of fungi for the samples of Sampling III

Sample	Code	Medium pH	Fungal Spore Count
liquid 30201	D-3A	5.5 3.5	2,333 per 100 mL 333 per 100 mL
liquid 38034	D-3B	5.5 3.5	1,167 per 100 mL <167 per 100 mL
liquid 32881	D-3C	5.5 3.5	<167 per 100 mL <167 per 100 mL
liquid 48047	D-3D	5.5 3.5	<167 per 100 mL <167 per 100 mL
muck 30201	D-3X	5.5 3.5	<333 per g <333 per g
muck 38034	D-3Y	5.5 3.5	<333 per g <333 per g
muck 46078	D-3Z	5.5 3.5	5,667 per g 4,333 per g

Table 13. The effect of Mertect on the growth of fifteen of the fungal isolates from Samplings II and III

Sample	Code	Relative Amount of Growth			
		Active Ingredient Conc. (ppm)			
		1	10	50	Control
muck	D-2A-1	++++	++	-	++++
muck	D-2A-2	++++	++++	++++	++++
muck	D-2A-3	++++	-	-	++++
muck	D-2A-5	++++	+	-	++++
muck	D-2A-8	++++	-	-	++++
muck	D-2A-9	+++	-	-	++++
slime	D-2B-1	++++	-	-	++++
slime	D-2B-2	++++	-	-	++++
slime	D-2B-3	++++	++++	-	++++
slime	D-2B-4	++++	++++	++++	++++
slime	D-2B-7	++++	-	-	++++
liquid 30201	D-3A-2	++++	++++	-	++++
muck 46078	D-3Z-1	+	-	-	++
muck 46078	D-3Z-3	++++	-	-	++++
muck 46078	D-3Z-4	++++	-	-	++++

+ 's indicate the relative amount of growth on plates, with
 ++++ being the maximum amount of growth observed

SESSION I: PAPER 3

ABSTRACT

I. Suzuki et al., University of Manitoba

We have carried out small laboratory column leaching experiments of a sulphide ore for extraction of Cu and Zn and used the information obtained to evaluate the feasibility of in situ underground bacterial leaching operation and its possible economics.

It is concluded that the ore is suitable for efficient bacterial leaching, but the effect of ore size and other factors relating to the scale-up have to be studied further before a more definite economic estimate can be made.

SESSION I: PRÉSENTATION 3

RÉSUMÉ

Nous avons effectué dans une petite colonne de laboratoire des expériences de lixiviation sur un minerai sulfuré, dans le but d'extraire Cu et Zn, et nous avons utilisé l'information obtenue pour évaluer la faisabilité d'une exploitation souterraine in situ par lixiviation bactérienne, et sa rentabilité économique éventuelle.

On en a conclu que le minerai se prêtait à une lixiviation bactérienne efficace, et qu'il fallait davantage étudier l'effet des dimensions granulométriques du minerai et d'autres facteurs en rapport avec la mise à l'échelle, avant de pouvoir formuler une estimation économique plus précise.

COMPLEX SULPHIDE BASE METAL LEACHING AND ECONOMICS

INTRODUCTION

We have been studying the possibility of leaching Cu and Zn from a complex sulphide ore using bacterial activities. The ore used was from the Flin Flon mine of the Hudson Bay Mining and Smelting Co. Ltd., Manitoba (HBM&S). We have carried out shake flask and column leaching experiments using a variety of Thiobacillus ferrooxidans strains and Thiobacillus thiooxidans strains isolated from mine sites. Our conclusion was that the most effective leaching was achieved by a combined action of selected strains of T. ferrooxidans and T. thiooxidans (1,2).

The extension of laboratory results to the in situ underground bacterial leaching involves many difficult problems (3,4). In this paper we will try to consider some of the problems and economic implications.

LABORATORY LEACHING STUDIES

Shake flask leaching studies of finely ground high grade ore (-200 mesh) containing 4.9% Cu, 12.5% Zn, 30% Fe and 37.5% S demonstrated that three T. ferrooxidans isolates (SM-1, SM-4 and SM-5) and two T. thiooxidans isolates (SM-6 and SM-7) were capable of leaching Cu and Zn effectively.

Column leaching studies of 3/4" ore (1.5-2 kg) with equal weight of sand in 7.5 x 59 cm (2.6 L) columns demonstrated that single strains of T. ferrooxidans or T. thiooxidans isolates leached 1% Cu and 2.5% Zn monthly on the average from the high grade ore, but 2.8% Cu and 3.3% Zn monthly from a low grade ore (0.81% Cu, 8.2% Zn, 30.5% Fe, and 43.3% S). The higher leaching rate with the low grade ore is encouraging because the low grade ore used is considered to be closer to the ore composition of the proposed in situ leaching site.

The highest leaching rate was achieved when a combination of T. ferrooxidans and T. thiooxidans was used on the low grade ore. The SM-7 and SM-4 combination produced the highest monthly rate of 5.6% Cu and 7.9 Zn extraction.

IN SITU UNDERGROUND LEACHING - FEASIBILITY

The following preliminary plan is based on the results of our laboratory column leaching experiments.

Leaching site

The area of the Flin Flon mine of HBM&S considered as Phase I is located between the 3,500 ft. level and 3,750 ft. level with a volume of $37,000 \text{ m}^3$ (1.3 million cubic feet) containing 15,000 tons of ore estimated to contain 179 tons Cu (1.17%) and 734 tons Zn (4.8%) as shown on Fig. 1. The area has the advantage of having the possibility for expansion in the following phases to 3.4 million cubic feet (40,000 tons of ore, 469 tons Cu and 1918 tons Zn) and eventually to the whole 3,000 ft. level - 3750 ft. level zone of 57 million cubic feet (500,000 tons ore) (Figs. 2 and 3). The site has been mined out and filled with sand and other materials. The remaining ore is considered to be 6-8 inches in diameter requiring no fragmentation costs. The Phase I leaching plan will be considered in detail below.

Ore particle size

One problem in the extension of small scale laboratory results to large scale in situ leaching is the size of ore particles. We have calculated the leaching rate with and without consideration of the ore size. The following description is based on the leaching operation considering the larger in situ ore size.

The size of ore is considered as ten times in diameter compared to the size of ore used in our laboratory column experiments ($3/4$ " in diameter), i.e. around 6-8 inches. When the ore size is ten times in diameter, the number of particles per m^3 or ton of ore is $1/1,000$ and the total surface area of a cubic

meter or ton of ore is 1/10 (Table 1, Fig.4). In percolation leaching the leaching solution will flow over the surface of particles. Thus, if we maintain the same volume of leaching solution per unit surface area, we should expect the same leaching rate per solution volume as the laboratory column experiments in the in situ leaching. The volume of leaching solution used in the laboratory experiments was 500 mL for 2,600 mL of ore-sand mixture. Therefore, the volume of leaching solution required in the in situ Phase I leaching plan is :

$$37,000 \text{ m}^3 \times 500 \text{ mL} / 2,600 \text{ mL} \times 1/10 = 712 \text{ m}^3.$$

At an estimated percolation rate of water in the leaching site of 10 inches/hour it will take 204 hours (170 ft. x 12 inches/ft + 10 inches/hour) to reach the bottom, giving a flow rate (pumping rate) of $3.5 \text{ m}^3/\text{hour}$ ($712 \text{ m}^3/204 \text{ hours}$).

Bacterial Leaching

In our best column leaching experiments we used 10% inoculum each of SM-7 and SM-4 cultures. We can achieve at best a 0.5% inoculum for SM-7 and 1% inoculum for SM-4 in this in situ leaching and hope for the further growth of bacteria during the leaching period.

As shown in Fig.5 the bacterial leaching operation will start with the growth of SM-7 on sulfur in the bioreactor at the 3,500 ft. level containing 3.5 m^3 of Starkey's No. 1 medium (5) at 5 cm depth covered with sulfur. SM-4 will be grown on Fe^{2+} in the bioreactor at the 3,750 ft. level containing 7 m^3 M9K medium

(1,6). The cells of SM-7 and SM-4 from 35 L of respective media will be used as inocula. SM-4 will be grown once more in 350 L of the medium for 5 days with aeration before its final inoculation into the bioreactor which will be aerated. After 10 days of growth at 20°C without agitation the SM-7 culture will be diluted with water to 35 m³ and will be piped out to the leaching area at the rate of 3.5 m³/hour. This is followed by the pumping of 7 m³ SM-4 culture from the bioreactor at the 3,750 ft. level up to the bioreactor at the 3,500 ft. level for piping out to the leaching area at 3.5 m³/hour.

Once all the inocular are gone the remaining 701.5 m³ of low phosphate (LP) medium (1,6) will be piped out at 3.5 m³/hour from the bioreactor at the 3,500 ft. level. The leaching medium coming out at the 3,750 ft. level will be recycled after the addition of H₂SO₄ to pH 2.3 if necessary for continuous leaching.

Leaching rate

The total ore in the phase I as described above is considered as 15,300 tons containing 179 tons Cu (1.17%) and 734 tons Zn (4.8%). Our column experiment with SM-7 and SM-4 with low grade ore (0.81% Cu, 8.2% Zn) gave a leaching rate of 5.6% Cu and 7.9% Zn per month. The average monthly leaching rates by single strains were 2.8% Cu and 3.3% Zn.

If it is assumed that the same percentage leaching rate as the column experiment is achieved in the in situ leaching, the monthly leaching rate will be 10 tons Cu (179 tons x 0.056) and

58 tons Zn (734 tons x 0.079) by SM-7 + SM-4 and 5 tons Cu and 24 tons Zn by single strains of bacteria.

If the particle size difference is considered as described above and the volume of leaching solution is reduced ten times to 712 m^3 to maintain the volume to surface area ratio of the column experiment, the monthly leaching rate may be calculated as 1 ton Cu and 5.8 tons Zn by SM-7 + SM-4 and 0.5 tons Cu and 2.4 tons Zn by single strains. This calculation is based on the assumption that the leaching takes place evenly on the surface of the ore particles and the rate may increase if the particles are leached unevenly due to mineral heterogeneity resulting in increased new surface areas.

Concentration of metals with ion exchange resin

One ton of Cu and 5.8 tons of Zn per 30 days means the daily production of 33.3 kg Cu and 193 kg Zn. If the underground concentration of Cu and Zn by cationic ion exchange resin is considered, 6.8 m^3 of the resin are required everyday ($226.3 \text{ kg} + 60 \text{ kg/m}^3 \times 1.8$), where 60 kg/m^3 is the approximate binding capacity of resin for Cu or Zn and 1.8 is the safety factor for other metals (Fe, Ca etc,) estimated from laboratory tests. For two alternating sets a total resin requirement will be 13.6 m^3 (486 cubic ft). Everyday a loaded set of 6.8 m^3 resin will be replaced with a new set and transported to surface for elution of metals with 6.8 m^3 4N H_2SO_4 (12%) and washing with water before being returned underground. The estimated concentration of Cu and Zn in the acid eluate will be 4.9 kg/m^3 or 4.9 g/L Cu

(33.3 kg + 6.8 m³) and 28.4 kg/m³ or 28.4 g/L Zn (193 kg + 6.8 m³), giving the concentration of Cu + Zn of 33.3 g/L.

Economics

It is not possible to make a definite economic estimate at this stage of study, but we can make some observations and recommendations for further study.

The area has the advantage of not requiring expensive fragmentation and the ores in the area are suitable for efficient bacterial leaching of both Cu and Zn, an important economic advantage compared to the consideration of Cu alone. The rate of in situ leaching may be calculated as 5.6% Cu and 7.9% Zn monthly using the best laboratory result SM-7 + SM-4) directly or 0.56% Cu and 0.79% Zn monthly with the consideration of ore surface area due to different ore sizes. Since these figures are based on theoretical approximations we will require a further study to examine the effect of ore size on the leaching rate before we can estimate more accurately the in situ leaching rate of Cu and Zn. This will involve not only experiments with different sizes of ore in small laboratory columns, but also a large column experiment with 6-8 inch ore particles, the same size as the estimated size in the in situ operation.

The concentration of metals with ion-exchange resins is effective, but expensive when large quantities of metals have to be handled. The estimated concentration of Cu + Zn in the acid eluate is only 33.3% g/L. In our laboratory column studies a higher concentration was often reached without any concentration procedures.

It may therefore be more economical to pump up the highly concentrated leaching solution to the surface for further treatment such as, e.g., solvent extraction. At the monthly leaching rate of 0.56% Cu and 0.79% Zn the concentration of Cu + Zn after one month will be $(1 \text{ ton Cu} + 5.8 \text{ tons Zn})/712 \text{ m}^3 = 1.4 \text{ g Cu/L} + 8.1 \text{ g Zn/L} = (\text{Cu} + \text{Zn})/\text{L}$. After 3 months of leaching the concentration would become $28.5 \text{ g (Cu} + \text{Zn)/L}$. If the leaching solution volume can be reduced further by using the factor: the solution volume/weight of ore instead of the solution volume/volume of leaching zone as described above, then the volume of leaching medium becomes $15,000 \text{ tons}/1.7 \text{ kg} \times 0.5 \text{ L} \times 1/10 = 441 \text{ m}^3$. The concentrations of Cu and Zn then become 2.3 g Cu/L and 13.2 Zn/L and $15.5 \text{ g (Cu} + \text{Zn)/L}$. It will take only two months to reach $31 \text{ g (Cu} + \text{Zn)}$ concentration.

It would be very interesting to see whether these high concentrations could actually be achieved in a large column with 6-8 inch ore. Of course the final proof of the leaching rate and economics will not be known until the in situ operation such as Phase I described here is actually carried out.

CONCLUSION

The sulphide ore at the Flin Flon mine of HBM&S is suitable for effective bacterial leaching of Cu and Zn with a combination of SM-7, T. thiooxidans and SM-4, T. ferrooxidans.

The design of in situ bacterial leaching operation is possible and the ore size difference between the laboratory column experiments and the in situ operation can be considered in theoretical approximation.

It is recommended that the effect of ore size on the leaching rate should be studied including the 6-8 inch ore to approximate the in situ condition, particularly to see whether a high (Cu + Zn) concentration obtained in the laboratory column could be achieved, a critical economic factor in the in situ operation.

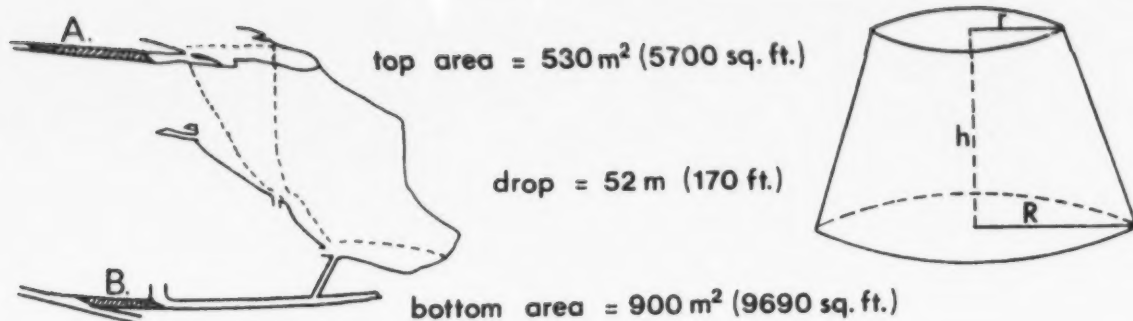
ACKNOWLEDGEMENTS

The authors wish to thank the Hudson Bay Mining and Smelting Co., Ltd. for the contract, as well as the Mines Branch of the Department of Energy and Mines of Manitoba and CANMET for making the contract possible under the Canada-Manitoba Mineral Development Agreement. They would also like to thank Mr. B. Barlin of HBM&S, Dr. R.G.L. McCready of CANMET, and Mr. d.c. Cook of Energy and Mines for their extensive help and co-operation. The authors also thank the NSERC for a Postgraduate Scholarship to H.M.L.

REFERENCES

1. Suzuki, I.; Lizama, H.M.; and Oh, J.K. "Bacterial leaching of a complex sulphide ore"; Biominet Proceedings; R.G.L. McCready, Editor; CANMET Special Publication SP86-9; pp. 127-146; 1987.
2. Lizama, H.M.; and Suzuki, I. "Bacterial leaching of a sulfide ore by Thiobacillus ferrooxidans and Thiobacillus thiooxidans. 1. Shake flask studies"; Biotechnology and Bioengineering; (accepted for publication); 1987.
3. Ismay, A.; Rosato, L.; and McKinnon, D. "Engineering preeasibility for in-place bacterial leaching of copper"; Fundamental and Applied Biohydrometallurgy; R.W. Lawrence, R.M.R. Branion, and H.G. Ebner, Editors; Elsevier; Amsterdam; pp.191-213; 1986.
4. Miller, P.C. "Large-scale bacterial leaching of a copper-zinc ore in situ"; Fundamental and Applied Biohydrometallurgy; R.W. Lawrence, R.M.R. Branion, and H.G. Edner, Editors; Elsevier, Amsterdam; pp. 215-239; 1986.
5. Starkey, R.L. "Concerning the physiology of Thiobacillus thiooxidans, and autotrophic bacterium oxidizing sulfur under acid conditions"; J. Bacteriol. 10: 135-163; 1925.
6. McCready, R.G.L.; Wadden, D.; and Marchbank, A. "Nutrient requirements for the in-place leaching of uranium by Thiobacillus ferrooxidans"; Hydrometallurgy 17: 61-71; 1986.

FIGURES



$$\text{volume} = \frac{1}{3}\pi h(R^2 + r^2 + Rr) = 37000 \text{ m}^3 \text{ (1.3 million cu. ft.)}$$

ore = 179 tons Cu, 734 tons Zn

Leaching solution : 712 m³ or 188090 US gallons

Pumping rate : 3.5 m³ per hour or 15 US gallons per minute

Bioreactors : A. at 3500 level - 116 m³
 B. at 3750 level - 208 m³

Fig. 1. Possible in situ leaching area (Phase I)

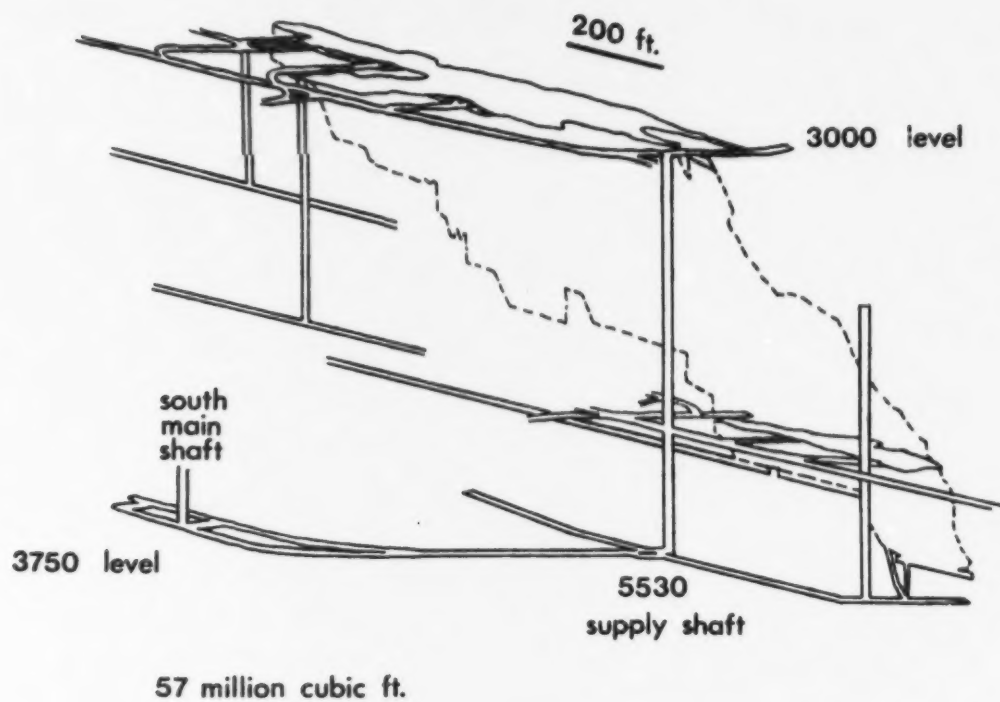


Fig. 2. Possible in situ leaching area (Phase 2)

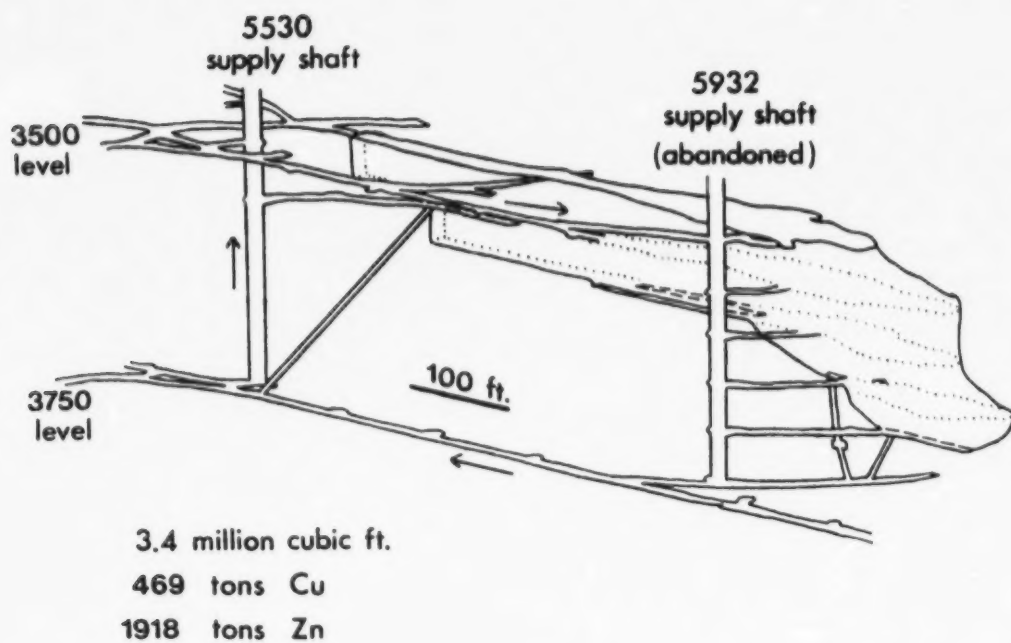
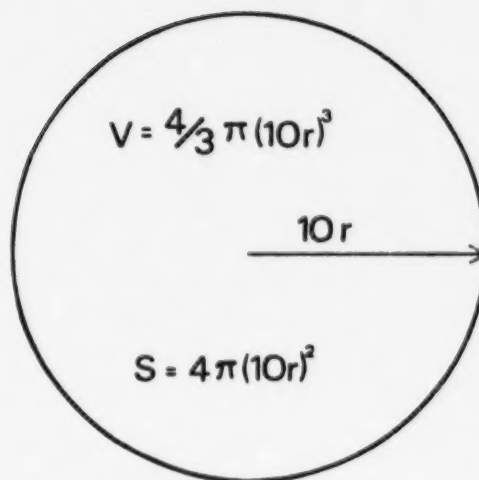


Fig. 3. Possible in situ leaching area (Phase 3)

$$V = \frac{4}{3} \pi r^3$$



$$S = 4 \pi r^2$$



$$V = \frac{4}{3} \pi (10r)^3$$

$$S = 4 \pi (10r)^2$$

Mass ratio = Volume ratio

$$= \frac{\frac{4}{3} \pi r^3}{\frac{4}{3} \pi (10r)^3} = \frac{1}{1000}$$

Surface area ratio of equal weights:

$$= \frac{1000 \text{ particles}}{1 \text{ particle}} \times \frac{4 \pi r^2}{4 \pi r^2 (10)^2} = \frac{1000}{100} = \frac{10}{1}$$

Fig. 4. Surface area ratio of equal weights of spherical particles with different diameters

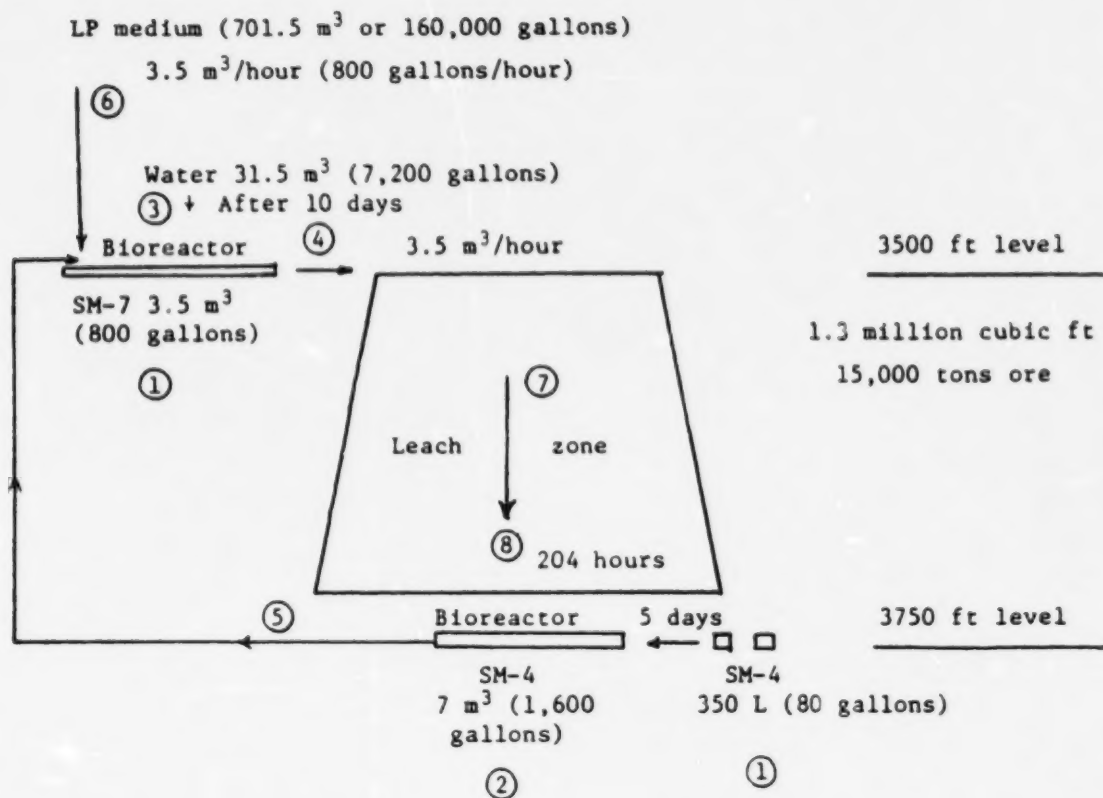


Fig. 5. Bacterial leaching design for Phase I

SESSION I: PAPER 4

Abstract

R.W. Lawrence, Coastech Research Inc.

A review of the work carried out on mineral leaching using the thermophilic bacterium Sulfolobus has indicated that the organism has some potential advantages as an alternative to the mesophilic bacterium Thiobacillus ferrooxidans for the pretreatment of refractory gold ores to enhance gold recovery. The results of an exploratory test program has not shown Sulfolobus to be a superior organism for oxidizing two refractory ores and four concentrates. Further work is required to gain experience in the development of a viable thermophilic system within practical operating constraints. An economic comparison between thermophilic and mesophilic systems has indicated that capital and operating cost savings may be realized by using Sulfolobus if rapid oxidation kinetics at high pulp density can be demonstrated.

SESSION I: PRÉSENTATION 4

POTENTIEL DES BACTÉRIES THERMOPHILES DANS LE PRÉTRAITEMENT DES MINÉRAIS AURIFÈRES RÉFRACTAIRES

R.W. Lawrence

Coastech Research Inc.
869 West Third Street
Vancouver nord (C.-B.) V7P 1E2

Résumé

Un examen des travaux effectués sur la lixiviation des minéraux au moyen de la bactérie thermophile Sulfolobus a révélé que l'organisme pourrait remplacer à certains égards la bactérie mésophile Thiobacillus ferrooxidans dans le prétraitement des minerais aurifères réfractaires visant à augmenter le taux de récupération d'or. Les résultats d'un programme d'essais exploratoires a montré que Sulfolobus n'était pas un organisme supérieur pour oxyder deux minerais réfractaires et quatre concentrés. D'autres travaux sont nécessaires pour acquérir plus d'expérience dans la mise au point d'un système thermophile viable sujet à des contraintes de fonctionnement pratique. Une comparaison, sur le plan économique, entre les systèmes thermophile et mésophile, a révélé que des économies pourraient être réalisées au chapitre des coûts d'immobilisation et d'exploitation par l'utilisation de Sulfolobus si l'on parvenait à faire la démonstration des étapes de la cinétique de l'oxydation rapide à une densité de pulpe élevée.

THE POTENTIAL OF THERMOPHILIC BACTERIA IN THE PRETREATMENT OF REFRACTORY GOLD ORES

Introduction

A nine-month laboratory study has been carried out on behalf of CANMET, Energy, Mines and Resources, Canada, to investigate the potential of using thermophilic bacteria, specifically Sulfolobus acidocaldarius, in a biological pretreatment process for refractory sulphidic gold ores and concentrates. In addition to a laboratory experimental program to determine critical process parameters, the study included a review of previous work involving the use of Sulfolobus in mineral leaching applications and a preliminary economic comparison between a thermophilic leaching system and a mesophilic system using the more extensively studied bacterium Thiobacillus ferrooxidans. This paper provides an overview of some of the key aspects of the study.

Biohydrometallurgical Pretreatment of Refractory Gold Ores

The use of bacteria in the pretreatment of refractory sulphidic gold ores and concentrates is gaining acceptance as a viable alternative technology to roasting and pressure oxidation. The basis of biological technology has been the capability of the bacterium Thiobacillus ferrooxidans to oxidize sulphide minerals and ferrous iron in acidic environments at practical rates. This microorganism is therefore able to break down gold-bearing sulphides such as pyrite and arsenopyrite to liberate the contained precious metals for recovery by cyanidation. Although there are currently no commercial biological oxidation plants to pretreat refractory gold materials in North America, a process using T. ferrooxidans has been successfully piloted on a 2 tpd scale at Equity Silver Mines, B.C. in 1984-85 (1). Small

commercial plants are also reportedly in operation in South Africa and Zimbabwe.

In addition to T. ferrooxidans, which has an optimum temperature range of 30-37°C, there are other microorganisms capable of oxidizing sulphide minerals and which may provide a suitable alternative to T. ferrooxidans for the treatment of refractory ores. Some of these organisms are moderately or extremely thermophilic, functioning at higher temperatures than the mesophilic T. ferrooxidans. Several recent review articles and technical papers can be found on this topic (2-4). One particular organism, the extremely thermophilic Sulfolobus, appears to have particular utility and is the subject of this study.

Sulfolobus

Sulfolobus was originally isolated and described as a new genus of bacteria by Brock and co-workers in 1972 (5). They found that the organism has generally spherical cells and is a facultative autotroph, able to grow both autotrophically on sulphur and heterotrophically on yeast extract. In the absence of other oxidizable substrates, Sulfolobus has also been shown to be able to oxidize large quantities of ferrous iron in hot, acid conditions (6). Sulfolobus and closely related species have been isolated from acidic thermal waters at Yellowstone Park (5,7), deep sea hot vents (8), and coal piles containing pyrite (9). Despite its restricted habitats, Sulfolobus is world-wide in its distribution. Wiegel and Ljundahl (3) list the validly published Sulfolobus organisms as S. acidocaldarius, S. brierleyi and S. solfataricus.

Brock and co-workers (5) found that the temperature range for growth was around 55-80°C with an optimum of 70-75°C. They originally defined the optimum pH range for growth between 2 and 3, although later described the discovery of a massive population

of Sulfolobus in a hot spring at pH 0.9 (8). Tolerance to low pH is an important requirement for practical application to the oxidation of high-sulphur ores at high pulp density.

Sulfolobus has been little studied with specific reference to its application in the pretreatment of refractory gold ores, although Hutchins and co-workers (10) have presented data on the possible treatment of both sulphidic and carbonaceous refractory ores. Much of the published work on Sulfolobus is related to the oxidation of copper sulphide minerals and the potential for the extraction of copper from mine wastes and flotation concentrates (11-16). Early interest in Sulfolobus also centered around its ability to oxidize molybdenum sulphide (11,13,14,17,18). More recently, work on oxidation by Sulfolobus of other sulphide minerals such as pyrite, pyrrhotite, pentlandite, covellite, sphalerite, millerite, and arsenopyrite has been described (19-21). Sulfolobus has also been proposed for use in the desulphurization of coal (22).

For Sulfolobus to be useful in a practical sulphide oxidation system requires demonstration of fast oxidation kinetics, capability for complete mineral oxidation, tolerance to low pH and high metal concentrations, potential for selective oxidation of gold-bearing sulphides (23), operation at high pulp density, and high gold recovery following biooxidation pretreatment.

The oxidation of pyrite at fast rates has been observed by Norris and Parrott (20) who claim that higher oxidation rates and metal yields are an advantage of thermophilic leaching. This view is supported by others who have found that Sulfolobus is more effective in sulphide mineral leaching than the mesophilic thiobacilli (12,16,18,24). Others (21) have found the mesophiles more suitable, but observed differences in the ability to leach sulphide minerals between strains of the same species. Marsh and

co-workers (19) also postulated that the rate and extent of mineral dissolution depends on the strain of Sulfolobus.

Norris and Parrott (20) found that Sulfolobus can tolerate greater acidity than T. ferrooxidans and that cation tolerance is similar to that of mesophiles and moderate thermophiles. Lindstrom and Gunneriuson (25) found the leaching of an arsenopyrite concentrate limited by soluble arsenic concentrations above 1.5 g/L. Work by Kargi and Robinson (22) on the desulphurization of coal showed that nutrient concentrations, particularly N/P and N/Mg ratios, affect oxidation rates of pyrite. In the same study, maximum pyrite oxidation was found to occur at pH 1.5. Oxidation rates were found to be proportional to pulp density up to 15% solids, with an optimum at 20% solids. No increase in rates was observed over 20%. Carbon dioxide addition did not affect pyritic sulphur removal rates at 10% pulp density, although increasing carbon dioxide may improve rates at higher pulp densities and /or pyrite concentrations. Other workers (4,8,20) have pointed to the beneficial effects of carbon dioxide addition.

The ability of Sulfolobus to oxidize sulphides rapidly at high pulp densities has not been demonstrated. P.R.Norris (personal communication) has obtained much faster pyrite oxidation rates for Sulfolobus over T. ferrooxidans at very low pulp densities but observes poor rates for the thermophile above 2% solids in stirred reactors. Problems with high density leaching may be related to the ability of some Sulfolobus strains to withstand the stress of an agitated system. Although some workers consider Sulfolobus strains as some of the most robust organisms for leaching (3,26), others have found that certain strains will grow autotrophically only in static culture (20).

In summary, Sulfolobus and related bacterial species has not been extensively studied for mineral oxidation applications but appears to have potential as an alternative organism to T.

ferrooxidans for the biological pretreatment of refractory gold ores. Significant experimental progress has been made and results indicate that higher oxidation rates and metal yields may be possible with the thermophilic system. The rapid oxidative ability of Sulfolobus in high-density pulps is, however, still in question. Low-sulphur coal pulps of up to 20% solids have been leached successfully but the oxidation of high-sulphur, high - density pulps has not been demonstrated.

Laboratory Study

A laboratory study has been carried out with the objective of evaluating the potential of developing a Sulfolobus-based oxidation system for the treatment of refractory gold ores within practical operating constraints. Investigations included studies of the effect of initial pH, agitation, pulp density, and carbon dioxide addition on several refractory ores and concentrates.

Organisms

The culture of Sulfolobus, probably S. acidocaldarius, isolated from a pyritic coal pile (9), was provided by P.R. Norris. T. ferrooxidans were obtained from Coastech stock cultures maintained on pyrite-arsenopyrite concentrates.

Ores and Concentrates

Four concentrate and two ore samples were used in Sulfolobus evaluation tests. All were refractory to cyanidation. Two of the concentrates and both ores contained pyrite as the dominant sulphide with small amounts of arsenopyrite. Two of the concentrates had high arsenopyrite contents. All ores and concentrates were ground to 75-90% passing 200 mesh. Table 1 provides head assay data for the test materials.

Growth Media, Leach Conditions and Assays

A growth medium containing (g/L) $(\text{NH}_4)_2\text{SO}_4$ (2.1), MgSO_4 (0.18), K_2HPO_4 (0.18), KCl (0.04), and $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (0.004) was used for Sulfolobus tests except where otherwise stated. For T. ferrooxidans, a medium containing (g/L) $(\text{NH}_4)_2\text{SO}_4$ (3.0), MgSO_4 (0.25), K_2HPO_4 (0.25), KCl (0.05), and $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (0.005) was used.

With the exception of one agitated tank leach test, all tests were carried out using various weights of ore or concentrate suspended in 50 mL of growth medium in 250 mL baffle-bottomed Erlenmeyer flasks. Pulp pH was stabilized with sulphuric acid at the required value prior to inoculation with a culture (4-10% by volume) previously grown on the test ore or concentrate. Flasks were incubated on reciprocating shakers at 33°C/150 rpm (T. ferrooxidans), and 64°C/120 rpm (Sulfolobus). Static Sulfolobus tests were conducted using a stationary incubator maintained at 64°C. Flasks incubated at the higher temperature were sealed with aluminum foil to minimize evaporation. Losses due to evaporation in all flasks were made up daily.

Tank leach tests were carried out in round 5L baffled plexiglas reactors agitated by a vari-speed turbine impellers. Temperature of the pulps was maintained at 69°C using an immersion heater. Air was introduced under the impellers at approximately 800 mL/min. Carbon dioxide was added to the air stream to one tank to give 1-5% by volume. Water was added frequently to make up for evaporation losses.

Flasks and tanks were monitored periodically for pH, Eh, and leached iron. Leached iron was routinely determined as soluble iron, Fe(S), by direct analysis of leachates by atomic absorption spectrophotometry. In addition, leached iron was periodically determined as total leached iron, Fe(T), by atomic absorption spectrophotometry following dissolution of iron which had precipitated under specific conditions of pH at the higher temperature. As much as 20% of the leached iron (ferric) was found to precipitate in tests at 64°C at pH's below 1.6. The presence of the nutrient salts also promoted a higher degree of precipitation.

Amenability of Ores/Concentrates to Sulfolobus Oxidation

The initial experimental plan was to perform preliminary shake-flask tests on three refractory concentrates and select the most amenable for use in the tests program. This plan did not proceed as expected as none of the initial concentrates evaluated (CIN, NN, MET) responded very favorably in terms of prolific bacterial growth or rate of pyrite oxidation, as measured by iron solubilization, in a reasonable time. Eventually, four concentrates and two ores were tested but, even after several serial transfers, no one material could be selected as being significantly more suitable than any other for exclusive evaluation.

Over the duration of the test program, all six ores and concentrates were used in several shake flask tests to evaluate the various process parameters. Table 2 provides a summary of the best Sulfolobus leach data obtained for each ore/concentrate. Typical leach data obtained for T. ferrooxidans leaches are shown for comparison. In all cases, the T. ferrooxidans data were superior to the Sulfolobus data. This is particularly the case for the two high-arsenic concentrates, MET and CP. In these two cases, inhibition of leaching by soluble arsenic may account for the poor performance.

Effect of Agitation

Figure 1 shows the change in pH (from sulphide oxidation) against time for agitated and static shake-flask leaches of NN, MET, and CIN concentrates at 4% solids. Sulphide oxidation took place in all flasks and at a faster rate with agitation. Figure 2 shows a comparison between shake-flask and agitated tank for the leaching of NN concentrate at 10% solids. The results of the two leaches were essentially the same and show that the improved leaching environment of an aerated, agitated tank did not improve rates, nor did the more violent agitation conditions of this system have a detrimental effect on rates.

Effect of Initial pH

The effect of initial pH on leaching performance was studied for NN and CIN concentrates. For NN concentrate at 5% solids, the initial pH had little effect on the rate of iron leached in the pH range 1.6 to 3.0 (Figure 3). The lower soluble iron concentrations for the tests initiated at pH 2.5 and 3.0 are probably due to iron precipitation at the higher pH rather than to a pH effect on kinetics.

Figure 4 shows the change in pH during leach tests on CIN concentrate at 5% solids initiated in the pH range 1.6 to 2.9. Again, the initial pH had little influence over the leach result, with all leaches terminating at a similar pH.

Effect of Nutrient Concentration

A series of ten leach tests was performed on NAM ore at 10% solids to evaluate the effect of nutrient composition on leach performance. The nutrient compositions were varied by changing the concentrations of $(\text{NH}_4)_2\text{SO}_4$, K_2HPO_4 and MgSO_4 as shown in Table 2. Concentrations of KCl and $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ were kept constant at 0.04 and 0.004 g/L respectively.

There was no significant difference in the quantity of iron leached in any of the tests after 25 days. This result is contrary to the findings of others (22) who showed that N/P and N/Mg ratios could have a marked effect on pyrite removal rates from coal using Sulfolobus.

Effect of Pulp Density

Figures 5 and 6 show the effect of pulp density (% solids) on the iron leach rate for NN and CIN concentrates using Sulfolobus and T. ferrooxidans. The results show that significantly higher oxidation rates were obtained for both concentrates with the mesophilic system. In addition, the extent of pyrite breakdown using T. ferrooxidans was much greater (see Table 2 for typical data for these two concentrates).

For leach systems using T. ferrooxidans we find that pulp densities around 30% solids are practical for ores. For concentrates, 20 -25% solids is usually employed to reduce the loading of metal and acid in the leachate. In large scale

application, the lower densities will also likely be necessary for high-sulphur feeds to reduce the heat produced per unit volume of pulp. The results obtained in this and other studies have not demonstrated that the Sulfolobus system can be operated at these practical densities.

Effect of Carbon Dioxide

The results of tank leach tests on NN concentrate conducted with and without the addition of carbon dioxide to the air supply are shown in Figure 7.

Initially, carbon dioxide was added at a rate to give approximately 5% by volume of the air sparged under the impeller. Iron leach rates in the tank with the CO₂ addition were lower than with air alone. Reducing the CO₂ content to approximately 1% at 525 hours did not impact the rate of leaching.

These results were not expected. As previously discussed, carbon dioxide addition has been found to be beneficial in improving pyrite oxidation rates and it was thought that the tank tests would have shown that the poor results obtained in the shake-flask work may have been due to a carbon dioxide limitation.

Comparative Economics

A preliminary economic comparison between a thermophilic and mesophilic leaching system for the pretreatment of a refractory gold ore has been carried out. Since the details of this comparison have been presented previously (27), only a summary of this part of the overall study will be presented here.

The basis of the comparison was a recent detailed engineering study carried out by Coastech for a T. ferrooxidans system to treat 350 tpd of a refractory gold ore grading 32 g/tonne Au. In the absence of conclusive data either from this or any other laboratory study, the comparison was made with the assumption that the process for the two systems would be essentially the same and that any differences in process details could be ignored for costing purposes. In addition it was assumed that the metallurgy and biohydrometallurgy of the two systems are equivalent.

On the basis of bench scale and pilot plant testwork conducted on the ore in our laboratory using the T. ferrooxidans system, a detailed process flowsheet was designed with the schematic arrangement shown in Figure 8 and the design criteria given in Table 3. The capital and operating costs for this base case processing plant are shown in Table 4. The costs shown are for the total plant (crusher to bullion) and for the biooxidation section only of the plant (bioleach tanks, solid-liquid separation, neutralization and cooling).

To compare the two systems it was assumed that the primary benefits to be derived from higher temperature operation would be reduced cooling costs and improved oxidation kinetics. For cooling, operation at the higher temperature would allow a downsizing of the cooling system. Figure 9 shows the effect of cooling water temperature on capital cost of the cooling system and indicates that costs for cooling in the mesophilic system could be double that of the thermophilic system. A similar graph could be drawn to represent the effect of cooling water temperature on operating costs. However, the difference in the costs of cooling expressed as a percentage of the overall costs shown in Table 4 are small at 1.6% of capital and 0.8% of operating for the most extreme case.

If improved oxidation kinetics can be obtained using Sulfolobus, then a reduction in capital costs in the bioleach section of the plant would be realized due to the reduction in retention time (Figure 10). Again, a similar relationship could be presented for kinetics and operating costs. Reduction of the retention time from the base case 60 hours to 30 hours would result in cost reductions, expressed as a percentage of overall costs, of 4.1% capital and 2.1 % operating. However, to realize such savings, the Sulfolobus system must be able to operate at comparable pulp densities to T. ferrooxidans. If not , potential cost savings due to faster kinetics are lost due to the increased cost of operating at a lower pulp density. This is shown for capital costs in Figure 11. A similar relationship exists between pulp density, residence time and operating costs.

Conclusions

The study of the thermophile Sulfolobus for mineral leaching applications is a relatively new field. However, despite some uncertainty in nomenclature and apparent differences in oxidative capability, a review of the literature shows that the organism clearly has utility and some workers have achieved significant experimental progress. Little work has been done to apply Sulfolobus to the treatment of refractory gold ores and concentrates. The ability of the organism to achieve rapid oxidation of goldbearing minerals such as pyrite has been claimed but considerable work is required to demonstrate that a thermophilic system is a viable alternative to the extensively studied mesophilic system using T. ferrooxidans under practical operating constraints.

The results of the current laboratory study did not show Sulfolobus to be a superior leaching organism to T. ferrooxidans. Four concentrates and two ores were evaluated but none provided iron leach rates or extractions as good as the mesophile. Further work is required to gain more experience in the use of Sulfolobus for the oxidation of refractory materials and to demonstrate fast oxidation kinetics at practical pulp densities. In addition, significant improvements in gold metallurgy following pretreatment by Sulfolobus has to demonstrated.

An economic comparison between a thermophilic and a mesophilic oxidation system for the pretreatment of a refractory ore has shown that kinetic improvements postulated for the higher temperature system can save significant capital and operating costs. However, the Sulfolobus-based system must be able to maintain comparable pulp densities to the T. ferrooxidans system to be competitive. Reduced cooling requirements for the thermophilic system offer only minor potential for improved process economics.

Acknowledgements

This work was carried out under contract with the Canada Centre for Mineral and Energy Technology (CANMET), Energy, Mines and Resources, Canada, and is funded under the Unsolicited Proposals Program of the Department of Supply and Services, contract no. 23440-6-9159/01-SQ.

The excellent technical assistance of S. Basra and A. Sadeghnohari is acknowledged.

References

1. Marchant, P.B. Commercial piloting and the economic feasibility of plant scale continuous biological tank leaching at Equity Silver Mines Limited. In: Fundamental and Applied Biohydrometallurgy, R.W. Lawrence, R.M.R. Branion and H.G. Ebner (Eds.), Elsevier, Amsterdam, 1986.
2. Brierley, C.L., Brierley, J.A., Norris, P.R. and Kelly, D.P. Metal-tolerant microorganisms of hot, acid environments. In: Microbial Growth and Survival in Extremes of Environment. Gould, G.W. and Corry, J.E.L. (Eds.), Academic Press, London, 1980.
3. Wiegel, J. and Ljundahl, L.G. The importance of thermophilic bacteria in biotechnology. Crit. Rev. Microbiol. 3 (1), 1986, 39-108.
4. Norris, P.R., Parrott, L.M. and Marsh, R.M. Moderately thermophilic mineral-oxidizing bacteria. In: Biotechnology for the Mining, Metal-Refining, and Fossil Fuel Processing Industries, Biotechnology and Bioengineering Symposium No. 16, H.L. Ehrlich and D.S. Holmes (Eds.), John Wiley & Sons, New York, 1986, 253-262.
5. Brock, T.D., Brock, K.M., Belly, R.T. and Weiss, R.L. Sulfolobus: a new genus of sulfur-oxidizing bacteria living at low pH and high temperature. Arch. Mikrobiol. 84, 1972, 54-68.
6. Brock, T.D., Cook, S., Petersen, S. and Mosser, J.L. Biogeochemistry and bacteriology of ferrous iron oxidation in geothermal habitats. Geochimica et Cosmochima Acta, 40, 1976, 493-500.
7. Brierley, C.L. and Brierley, J.A. A chemoautotrophic and thermophilic microorganism isolated from an acid hot spring. Can. J. Microbiol. 19, 1973, 183-188.
8. Brock, T.D. Thermophilic Microorganisms and Life at High Temperature. Springer-Verlag, New York, 1978.
9. Marsh, R.M. and Norris, P.R. The isolation of some

- thermophilic, autotrophic, iron- and sulphuroxidizing bacteria. FEMS Microbiol. Lett. 17, 1983, 311-315.
10. Hutchins, S.R, Brierley, J.A. and Brierley, C.L. Microbial pretreatment of carbonaceous gold ores. 116th Annual Meeting AIME, Denver, Feb 23-27, 1987.
11. Brierley, C.L. Thermophilic microorganisms in extraction of metals from ores. Develop. Industrial Microbiol., 18, 1977, 273-284.
12. Chakraborti, N. and Murr, L.E. Kinetics of leaching chalcopyrite-bearing waste rock with thermophilic and mesophilic bacteria. Hydromet. 5, 1980, 337-354.
13. Brierley, J.A. and Lockwood, S.J. The occurrence of thermophilic iron-oxidizing bacteria in a copper leaching system. FEMS Microbiol. Lett. 2, 1977, 163-165.
14. Brierley, J.A. and Brierley, C.L. Microbial leaching of copper at ambient and elevated temperatures. In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena. L.E. Murr, A.E. Torma and J.A. Brierley (Eds.), Academic Press, New York, 1978, 477-490.
15. Brierley, C.L. Leaching of chalcopyrite ore using Sulfolobus species. Devel. Indust. Microbiol. 21, 1980, 435-444.
16. Madsen, B.W. and Groves, R.D. Percolation leaching of a chalcopyrite-bearing ore at ambient and elevated temperatures with bacteria. USBM R.I. 8827, 1983, 18pp.
17. Brierley, C.L. Leaching, : use of a hightemperature microbe. Proc. Solution Mining Symp., Aplan, F.F., McKinney, W.A. and Pernichele, A.D. (Eds.), 1974, 461-469.
18. Brierley, C.L. Molybdenite-leaching: use of a high temperature microbe. J. Less Common Metals, 36, 1974, 237-247.
19. Marsh, R.M., Norris, P.R. and Le Roux, N.W. Growth and mineral oxidation studies with Sulfolobus. In: Recent Progress in Biohydrometallurgy, G. Rossi and A.E. Torma (Eds.), Assoc. Mineraria Sarda, 09016 Iglesias, Italy, 1983, 71-81.

20. Norris, P.R. and Parrott, L. High temperature, mineral concentrate dissolution with Sulfolobus. In: Fundamental and Applied Biohydrometallurgy, R.W. Lawrence, R.M.R. Branion and H.G. Ebner (Eds.), Elsevier, Amsterdam, 1986, 53-76.
21. Groudeva, V.I., Groudev, S.N. and Markov, K.I. A comparison between mesophilic and thermophilic bacteria with respect to their ability to leach sulphide minerals. In: Fundamental and Applied Biohydrometallurgy, R.W. Lawrence, R.M.R. Branion and H.G. Ebner (Eds.), Elsevier, Amsterdam, 1986, 484-485.
22. Kargi, F. and Robinson, J.M. Biological removal of pyritic sulphur from coal by the thermophilic organism Sulfolobus acidocaldarius. Biotech. Bioeng. 27, 1985, 41-49.
23. Lawrence, R.W. and Marchant, P.B. Biochemical pretreatment in arsenical gold ore processing. To be published in: Arsenic Metallurgy - Fundamentals and Applications, R.Reddy (Ed), TMS-AIME, Warrendale, PA., 1988.
24. Brierley, C.L. and Murr, L.E. Leaching: use of a thermophilic and chemoautotrophic microbe. Science, 179, 1973, 488-490.
25. Lindstrom E.B. and Gunneriuson, L. Semicontinuous leaching of arsenopyrite with a Sulfolobus strain at 70C, presented at 7th International Symposium on Biohydrometallurgy, Univ. of Warwick, UK, July, 1987.
26. Weiss, R.L. Subunit cell wall of Sulfolobus acidocaldarius. J. Bacteriol. 118, 1974, 275-284.
27. Lawrence, R.W. and Marchant, P.B. Comparison of mesophilic and thermophilic oxidation for the treatment of refractory gold ores and concentrates. In: Biohydrometallurgy 87, P.R. Norris and D.P. Kelly (Eds), Science and Technology Letters, Kew, England, in press.

TABLES

Table 1. Ore/Concentrate Head Assays

SAMPLE	Au g/t	Ag g/t	Fe %	As %	S %
Concentrates:					
NN	23.2	222.5	36.3	0.8	42.6
CIN	19.6	29.5	10.3	0.1	10.4
MET	26.0	35.0	40.0	12.0	41.0
CP	98.7	34.9	32.4	11.0	30.6
Ores:					
NAM	31.3	40.3	4.1	0.1	4.4
RD	7.2	59.0	9.5	0.1	11.1

Table 2. Comparison of *sulfolobus* and *T. ferrooxidans* Leach Data

SAMPLE	SULFOLOBUS			T. FERROOXIDANS		
	Pulp Density (% Solids)	Fe Extn (%)	Leach Time* (days)	Pulp Density (% Solids)	Fe Extn (%)	Leach Time* (days)
CIN	5	30	14	5	68	9
	10	25	14	10	60	7
	20	24	16	20	67	14
NN	5	27	28	5	77	17
	10	30	20	10	77	17
	20	25	20	20	35	17
	30	34	37			
MET	5	8	13			
	10	5	13	10	95	8
	20	4	17			
CP	5	23	9			
	10	31	52	10	95	7
NAM	5	36	9			
	10	57	20			
	15	52	27	20	90	4
	20	40	20	30	90	6
RD	6	46	52	26	54	3
				26	76	5

Table 3. Nutrient Concentrations for Leach Tests on NAM Ore

Test #	(NH ₄) ₂ SO ₄ (g/L)	K ₂ SO ₄ (g/L)	MgSO ₄ (g/L)	N/P	N/Mg
1	2.10	0.18	0.18	1.5	31.8
2	11.69	0.25	2.13	50.0	15.0
3	11.69	0.25	1.06	50.0	30.0
4	7.01	0.25	0.48	30.0	40.0
5	7.01	0.25	1.28	30.0	15.0
6	3.51	0.25	0.32	15.0	30.0
7	3.51	0.25	1.28	15.0	7.5
8	1.87	0.25	0.26	8.0	20.0
9	1.87	0.25	1.28	8.0	4.0
10	3.00	0.50	0.50	6.4	16.4

Table 4. 350 tpd Bioleach Plant: Base Case Design Criteria

FEED RATE, tpd	350
CIRCUIT AVAILABILITY, %	90
SULPHUR ASSAY, %	5
SULPHUR OXIDATION, %	70
RESIDENCE TIME, h	60
PULP DENSITY, %	30
NET HEAT, KW	2784
COOLING WATER, °C	10
MESOPHILIC SYSTEM, °C	40
THERMOPHILIC SYSTEM °C	70

Table 5. 350 tpd Bioleach Plant: Base Case Costs

PROCESS PLANT:

CAPITAL COST	\$7,442,000
OPERATING COST	\$32,66/t (Process)
	\$19.69/t (Labour)

BIOLEACHING:

CAPITAL COST	\$1,270,000	(17.1%)
OPERATING COST	\$17.83/t	(54.6%)
	\$ 0.99/t	(5.1%)

FIGURES

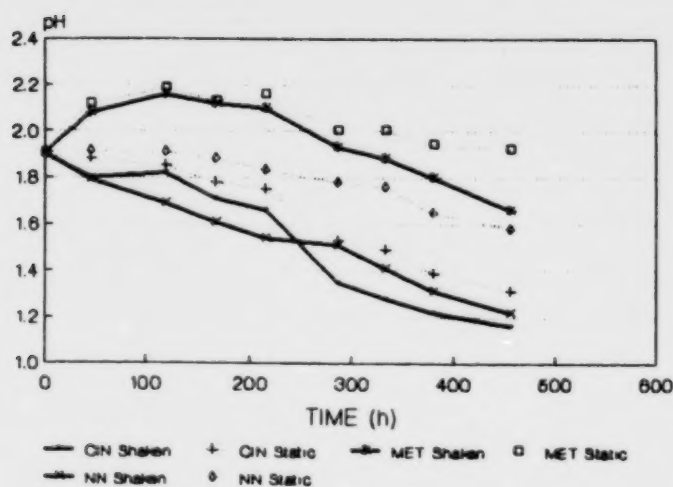


Fig. 1. The effect of agitation on the leaching of concentrates with *Sulfolobus*

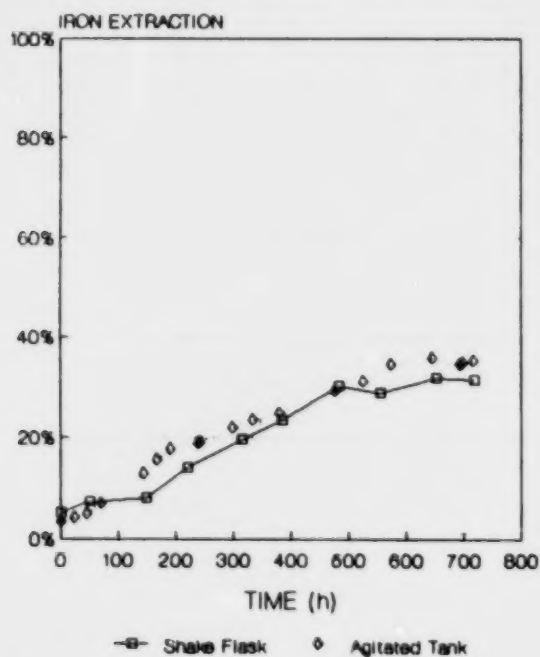


Fig.2. Leaching of NN concentrate in shake flask and agitated tank with *Sulfolobus*

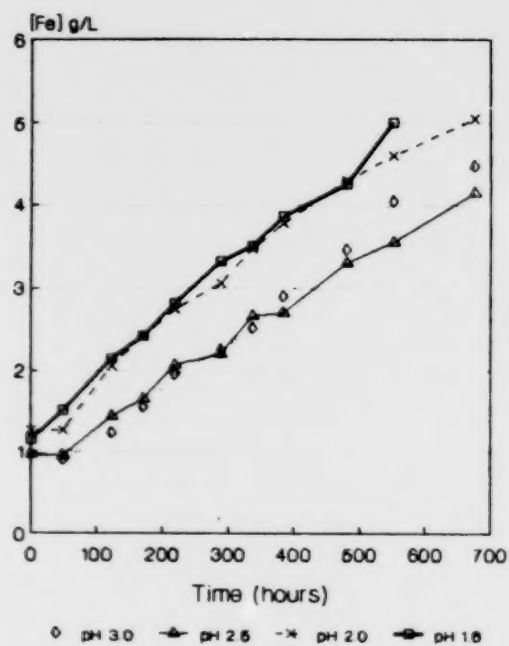


Fig. 3. The effect of initial pH on the leaching of iron from NN concentrate with Sulfolobus

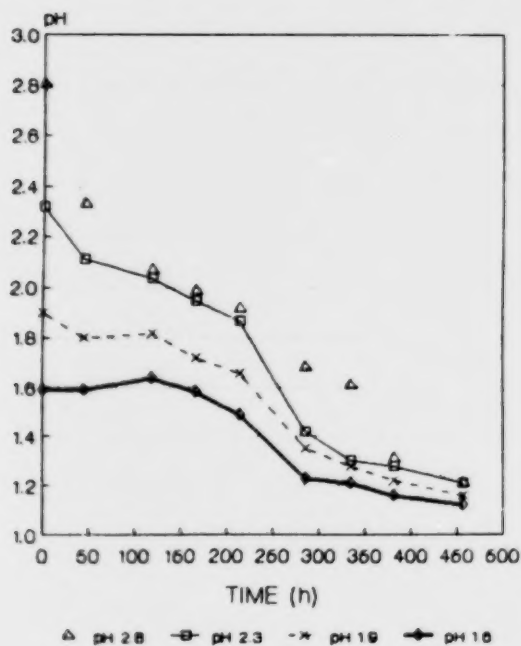


Fig.4. The effect of initial pH on the leaching of CIN concentrate with Sulfolobus

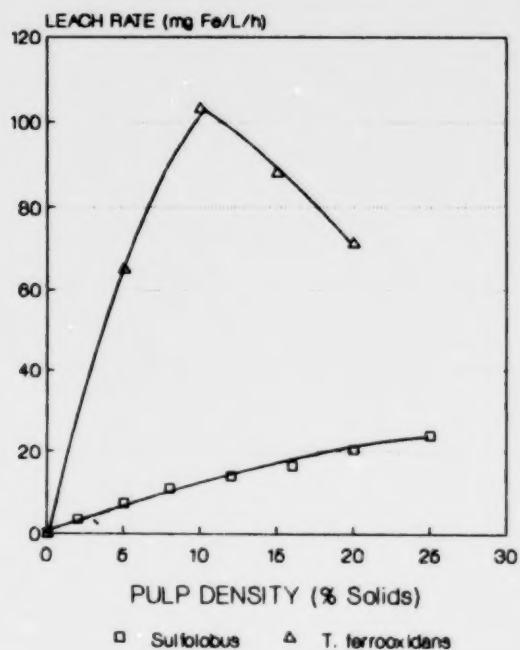


Fig. 5. The effect of pulp density on the leaching of NN concentrate with Sulfolobus

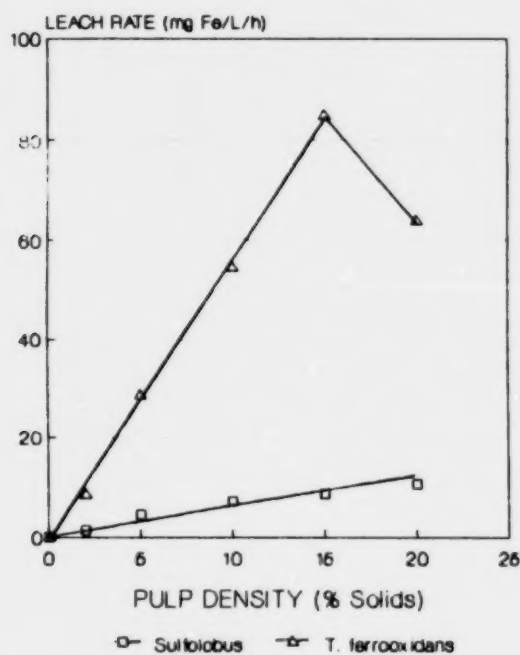


Fig.6. The effect of pulp density on the leaching of CIN concentrate with Sulfolobus

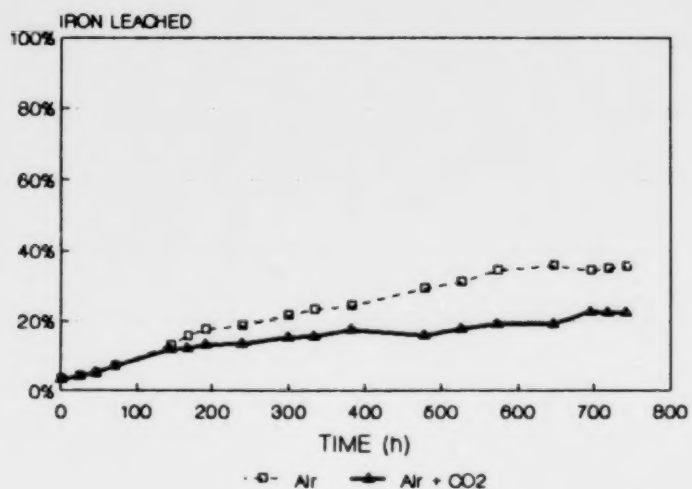


Fig. 7. The effect of carbon dioxide addition on the leaching of NN concentrate with Sulfolobus

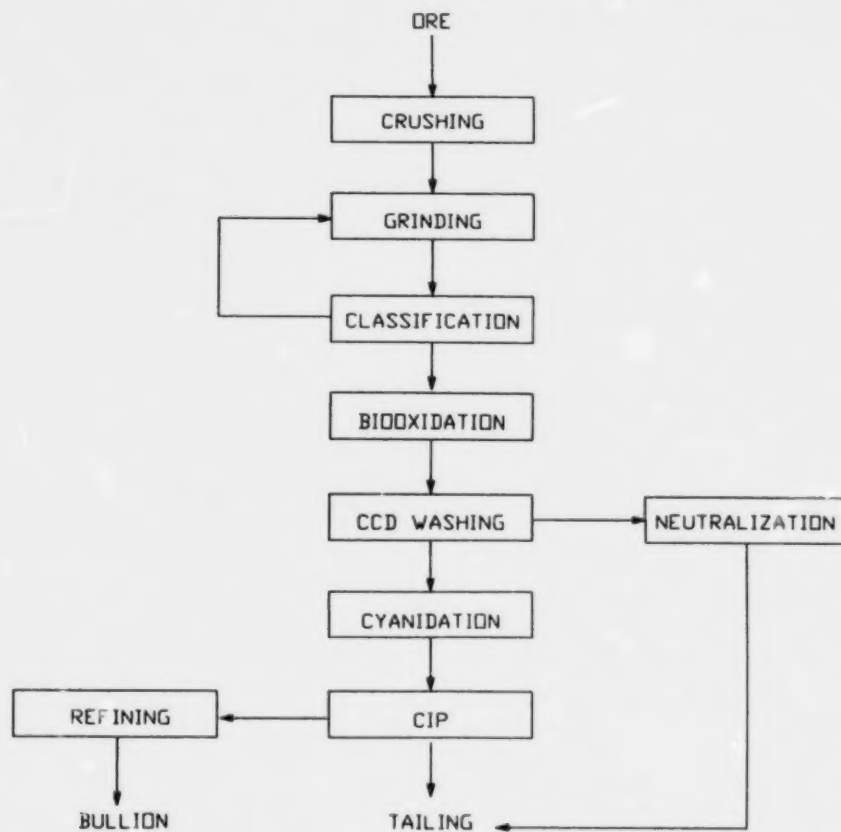


Fig.8. Schematic flowsheet for biooxidation process

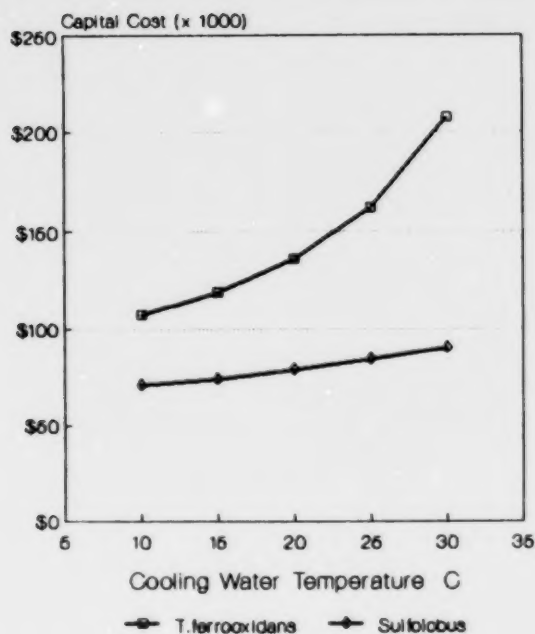


Fig. 9. The effect of cooling water temperature on capital cost of cooling system

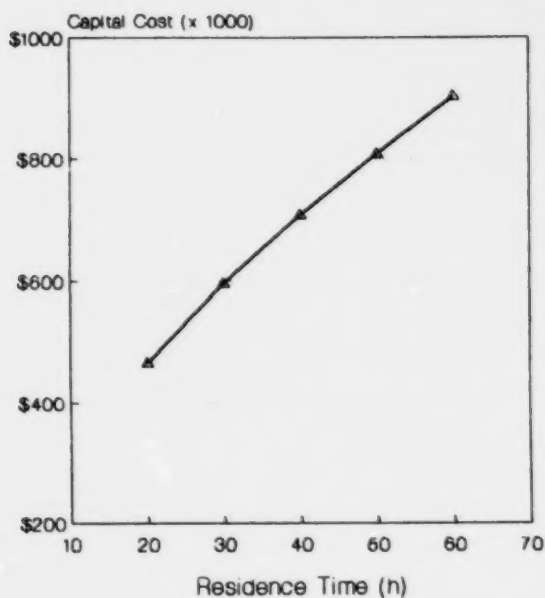


Fig.10. The effect of oxidation kinetics (residence time) on the capital cost of biooxidation plant at 30% solids

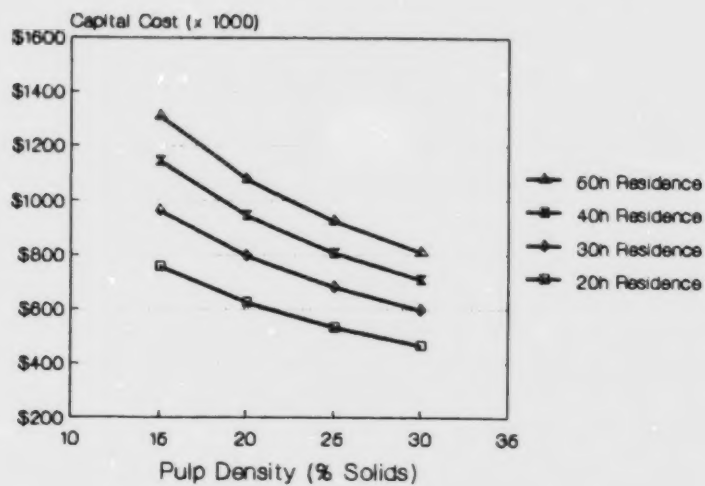


Fig. 11. The effect of oxidation kinetics and pulp density on the capital cost of the biooxidation plant

SESSION I: PAPER 5

V. Sanmugasunderam, W.D. Gould, R.G.L. McCready, S. Rajan,
M. Beaulne, and P. Mainwaring.

ABSTRACT

R.G.L. McCready, J. Salley, D.G. Hoppe and

D.R. Bartlett,

CANMET and Witteck

Polished discs of a complex sulphide ore were leached with three lixiviants; a mixed culture of Thiobacillus ferrooxidans and Thiobacillus thiooxidans, Thiobacillus ferrooxidans alone, and ferric sulphate. At either one or two week intervals the discs were examined by scanning electron microscopy, and the amount of soluble metals released from the discs were determined. The mixed culture solubilized more Cu, Zn and Pb than did either ferric sulphate or T. ferrooxidans alone. However, no differences in the rate of mineral dissolution were observed between the three treatments by scanning electron microscopy. After 1, 2 and 8 weeks most of the pyrrhotite, cubanite and sphalerite respectively had been leached from the surfaces of the discs.

Keywords: bacterial leaching, chemical leaching, complex base metal sulphide ores, preferential leaching, SEM.

SESSION I: PRÉSENTATION 5

V. Sanmugasunderam, W.D. Gould, R.G.L. McCready, S. Rajan
M. Beaulne, et P. Mainwaring

RÉSUMÉ

Des disques polis d'un minéral sulfuré complexe ont été lixiviés au moyen de trois produits de lixiviation; une culture mixte de Thiobacillus ferrooxidans et de Thiobacillus thiooxidans, Thiobacillus ferrooxidans employé seul, et du sulfate ferrique. Les disques ont été examinés, soit à une ou deux semaines d'intervalle, au moyen d'un microscope électronique à balayage, en vue de déterminer la quantité de métaux solubles qui s'en dégageaient. La culture mixte a dissous une plus grande quantité de Cu, Zn et Pb que la sulfure de fer ou T. ferrooxidans employé seul. Toutefois, l'analyse au microscope électronique à balayage n'a révélé aucune différence entre les trois traitements quant au taux de dissolution des minéraux. Après une, deux ou huit semaines, la plus grande partie de la pyrrhotine, de la cubanite et de la sphalérite respectivement, qui se trouvaient à la surface des disques, avait été lixiviée.

Mots-clé : lixiviation microbiologique, lixiviation chimique, minerais complexes sulfurés provenant de métaux communs, lixiviation préférentielle. microscopie électronique à balayage (SEM).

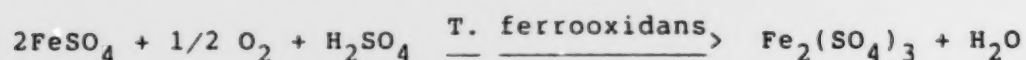
BACTERIAL AND CHEMICAL LEACHING OF COMPLEX SULPHIDE ORES

INTRODUCTION

Bacterial leaching of copper using Thiobacillus ferrooxidans has been practiced for quite some time (Brierley, 1978). More recently, uranium has also been extracted from pyritic ores due to oxidation of the pyrite and subsequent oxidation of U^{4+} to U^{6+} (Brierley, 1978, McCready et al, 1986). There has been considerable research to characterize the nutritional requirements, physiological properties, and the mechanism of attack on sulphide minerals by Thiobacillus ferrooxidans (Ralph, 1979).

The dissolution and oxidation of sulphide minerals by T. ferrooxidans can occur by two processes simultaneously (Brierley, 1978):

1. Bacterial oxidation of ferrous ions to ferric ions, which in turn oxidize the metal sulphide (MS):



2. Direct bacterial oxidation of both the metal sulphide and the elemental sulphur formed from chemical attack to produce sulphuric acid and soluble metal ions. Silverman (1967) postulated several mechanisms for direct oxidation that required attachment of the organisms to the mineral surfaces. Bacterial

attachment to mineral surfaces has been observed for a number of systems (Bennett and Tributsch, 1978; Ehrlich and Fox, 1967; Gormley and Duncan, 1974; Murr and Berry, 1976). Murr and Berry (1976) using a scanning electron microscope observed preferential attachment of Caldariella sp. to the pyritic and other sulphidic phases of a low-grade copper ore. They also found preferential bacterial attachment to specific areas on turquoise. Bennett and Tributsch (1978) leached pyrite crystals in stationary cultures of T. ferrooxidans. They examined the crystals using scanning electron microscopy, and found the leached zone to correspond with bound bacteria and as well as fracture lines and dislocations in the crystal structure. There is at present no direct proof for any of the mechanisms postulated for bacterial attachment and attack of sulphide minerals. There have also been no studies that have elucidated the contribution of chemical (Fe^{3+}) and direct bacterial oxidation to the dissolution and oxidation of sulphide minerals.

The objective of this study are:

1. to observe preferential attachment of T. ferrooxidans to the surface of a complex sulphide ore; and
2. to compare the susceptibility of various minerals in a complex sulphide ore to chemical (ferric sulphate) and bacterial leaching.

Materials and Methods

The ore samples were obtained from the Flin Flon Mine of Hudson Bay Mining and Smelting.

The essential mineralogy of the Flin Flon ore is simple. The dominant opaque minerals are pyrite, pyrrhotite, chalcopyrite, sphalerite, and galena. The sphalerite coexists with pyrite and pyrrhotite. The gangue minerals are those of the host rock which is composed of quartz-rich gneiss with small interlayers of calc silicate gneiss: quartz, plagioclase, biotite, almandine, hornblende, clinopyroxene. The chemical composition of the ore is given in Table 1. The samples selected for each treatment (eg. leaching with T. ferrooxidans) had similar mineralogical compositions.

Sections of hand-picked samples, containing metallic sulphides of interest, were embedded in an appropriate medium (epoxy resin). The mounted specimens were cylindrical in shape with the circular face having a diameter of 30 mm and the height of the cylinder being between 12 and 15 mm. After mounting, the samples were ground using successively finer grades of abrasive to achieve macroscopically flat surfaces.

Before examination by scanning electron microscopy, the specimen was coated with carbon to ensure electrical conductivity. The coating of the specimen minimizes problems due

to heating and build-up of surface charge during electron microscopy.

Ten polished discs were selected for the experiment. The discs were submerged in 100 mL (medium plus inoculum) of an autotrophic medium of the following composition: K_2HPO_4 0.1mM, $MgSO_4$ 0.5mM, $(NH_4)_2SO_4$ 0.5 mM, $FeSO_4$ 10mM, and an initial pH value of 2.5. Three of the discs were leached with a culture of Thiobacillus ferrooxidans (E.L. 5, McCready et al , 1986), three discs were leached with a mixed culture of T. ferrooxidans and T. thiooxidans and three discs were leached with the same medium in which ferric sulphate replaced ferrous sulphate. A control disc was also immersed in sterile growth medium. Thymol was added as a bacteriostatic agent to the control treatment and the three discs leached with ferric sulphate solution. The pH was adjusted to 1.7 for the ferric sulphate lixiviant and the control in order to prevent the precipitation of iron salts (i.e. jarosites). Ten mL of an exponential phase culture of either T. ferrooxidans or the mixed culture were added to inoculate each treatment. The flasks were weighed at appropriate intervals in order to add sufficient distilled water to compensate for evaporative losses.

The discs were removed every week for the first four weeks, and at two week intervals for the duration of the experiment. The original medium and acidified lixiviant were analyzed for copper, zinc, lead, and nickel. The pH, and the concentration of ferric and ferrous iron were also monitored. The discs were

rinsed with distilled water, dried , lightly polished, and then both mineralogical analysis and scanning electron micrographs were taken in order to determine the sequence in which the different minerals were leached. Electron microscopy examination was carried out on two areas per disc were examined on ten of the discs (three discs per treatment and the control) for mineralogical composition. X-ray micrographs for iron, copper and zinc at K-alpha peak position were taken on two discs (disc leached with T. ferrooxidans and one with the mixed culture).

When the discs are returned after mineralogical analysis, they are washed thoroughly and sonicated for 15 minutes in an ultrasonic cleaner with a detergent. After sonication the discs are washed well, followed by distilled water rinse and then placed in freshly prepared medium for inoculation.

Results

The mixed culture of T. ferrooxidans and T. thiooxidans solubilized the greatest amounts of copper, lead and zinc from the ore (Figures 1, 2 and 3; Tables 2, 3 and 4). T. ferrooxidans was slightly more effective than ferric sulphate in leaching copper and zinc from the ore (Figures 1 and 2). Ferric sulphate was more effective than T. ferrooxidans in solubilizing lead. T. ferrooxidans and ferric sulphate were both more

effective than the mixed culture in solubilizing nickel (Figure 4; Table 5). After six weeks of incubation the rate of copper leaching in the presence of the mixed culture increased markedly (Figure 1).

Mineralogical examination of the ore discs at various time intervals did not reveal any obvious differences between the three treatments. After 1, 2 and 8 weeks most of the pyrrhotite, cubanite, and sphalerite, respectively had been leached from the surfaces of the discs. However, the pyrite and chalcopyrite remained unaltered after 14 weeks.

Discussion

The lack of correlation between the disappearance of sulphide minerals as visualized by electron microscopy and the solubilization of metals could be due to several reasons. The minerals occur in a three-dimensional matrix, and once dissolution has penetrated below the surface it is not possible to observe subsequent mineral degradation. Also, qualitative observations of the disappearance of a particular mineral are not definitive enough to correlate with the dissolution data. The data for iron concentrations in solution were not presented since it is impossible to correlate iron levels with the leaching rates of iron-containing minerals. The lixiviant initially contains 10 mM FeSO_4 (or $\text{Fe}_2(\text{SO}_4)_3$), and iron is also removed from solution by precipitation as jarosite during leaching. Although leaching rates can be calculated from metal solubilization data, a mass

balance cannot be calculated due to the heterogeneity of the discs. The very rapid dissolution of pyrrhotite during this experiment is consistent with the low stability of this mineral under oxidizing conditions.

If chemical oxidation of sulphide minerals by ferric ions were the only mechanism of attack a layer of sulphur would accumulate on the surface of the mineral and retard dissolution. Bacterial oxidation of the elemental sulphur would expose fresh mineral surfaces to attack. The higher leaching rates observed for three of the metals with the mixed culture is consistent with this hypothesis. The electrochemical stability of the various sulphide minerals, the ability of bacteria to bind to sulphide minerals and the geometry of the minerals in the rock matrix will affect the rate of bacterial leaching.

These leaching studies will continue until both the pyrite and chalcopyrite have been leached from the discs. Since it appears that most of the pyrrhotite had leached during the first week, future experiments will also examine the initial stages of the pyrrhotite dissolution.

TABLES

TABLE 1

Chemical Analysis of Flin Flon Mine Ore

Total C as CO ₃	0.53 %
Sulphur	22.46 %
SiO ₂	32.15 %
Nickel	<0.01 %
Copper	0.38 %
Iron	19.53 %
Zinc	10.54 %
Lead	0.11 %
Magnesium	0.46 %
Aluminum	3.34 %
Calcium	0.28 %
Gold	5.3 ppm
Silver	23.6 ppm

TABLE 2

Cumulative Extraction of Copper from the Complex Sulphide Ore Discs by Various Treatments

Week	Amount of Copper extracted in mg		
	<u>Thiobacillus ferrooxidans</u>	Mixed Culture	Ferric
1	0.06	0.20	0.04
2	0.19	0.40	0.06
3	0.31	0.56	0.08
4	0.36	0.62	0.11
6	0.59	1.29	0.16
8	0.86	2.36	0.22
10	1.02	2.66	0.26
12	1.29	3.02	0.32
14	1.66	3.22	0.37
16	2.06	4.39	0.44

TABLE 3

Cumulative Extraction of Lead from the Complex Sulphide Ore
Discs by Various Treatments

Week	Amount of Lead extracted in mg		
	<u>Thiobacillus ferrooxidans</u>	Mixed Culture	Ferric

1	0.04	0.05	0.06
2	0.07	0.08	0.10
3	0.11	0.11	0.12
4	0.14	0.14	0.15
6	0.18	0.28	0.18
8	0.24	0.44	0.24
10	0.29	0.61	0.27
12	0.36	0.69	0.35
14	0.36	0.82	0.42
16	0.40	0.87	0.45

TABLE 4

Cumulative Extraction of Zinc from the Complex Sulphide Ore
Discs by Various Treatments

Week	Amount of Zinc extracted in mg		
	<u>Thiobacillus ferrooxidans</u>	Mixed Culture	Ferric

1	0.02	0.03	0.03
2	0.03	0.04	0.04
3	0.04	0.05	0.05
4	0.05	0.06	0.07
6	0.06	0.10	0.09
8	0.08	0.15	0.11
10	0.10	0.17	0.12
12	0.12	0.21	0.15
14	0.17	0.27	0.18
16	0.27	0.37	0

TABLE 5

Cumulative Extraction of Nickel from the Complex Sulphide Ore
Discs by Various Treatments

Week	Amount of Nickel extracted in mg		
	<u>Thiobacillus ferrooxidans</u>	Mixed Culture	Ferric
1	0.03	0.03	0.08
2	0.05	0.04	0.10
3	0.07	0.06	0.12
4	0.08	0.07	0.14
6	0.09	0.08	0.17
8	0.10	0.09	0.19
10	0.12	0.11	0.21
12	0.13	0.12	0.24
14	0.15	0.14	0.29
16	0.16	0.16	0.31

FIGURES

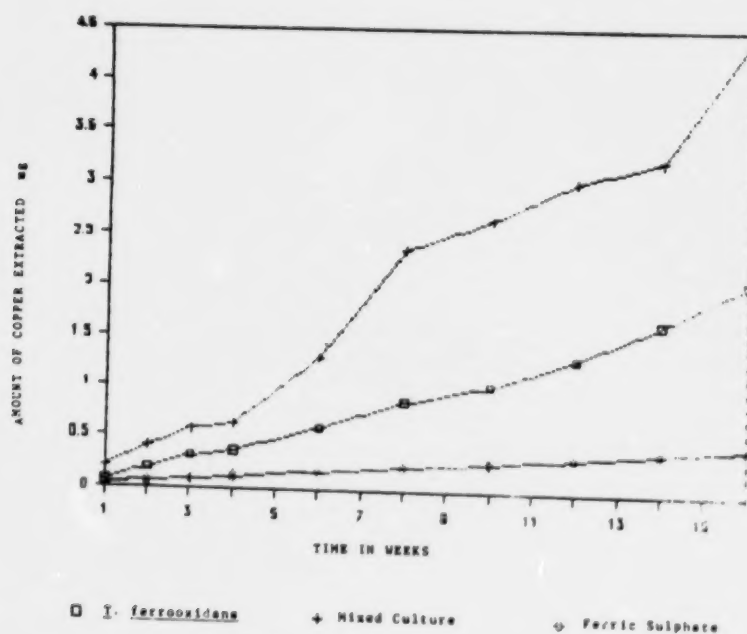


Fig. 1 Cumulative Extraction of Copper from Complex Sulphide Ore Discs by Various Treatments

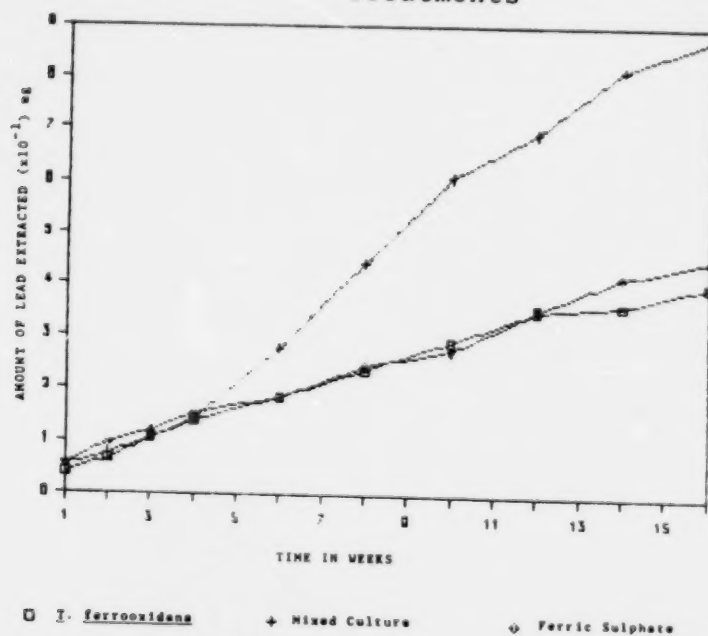


Fig. 2 Cumulative Extraction of Lead from Complex Sulphide Ore discs by various Treatments

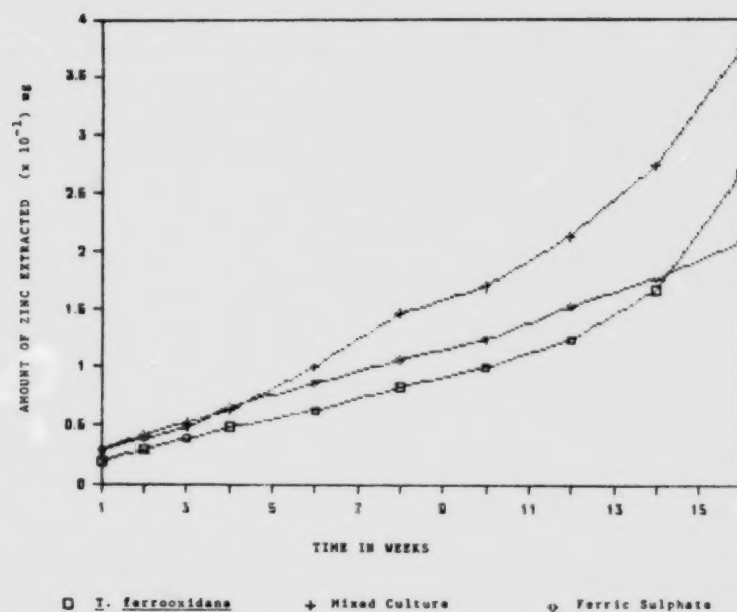


Fig. 3 Cumulative Extraction of Zinc from Complex Sulphide Ore Disc by various Treatments

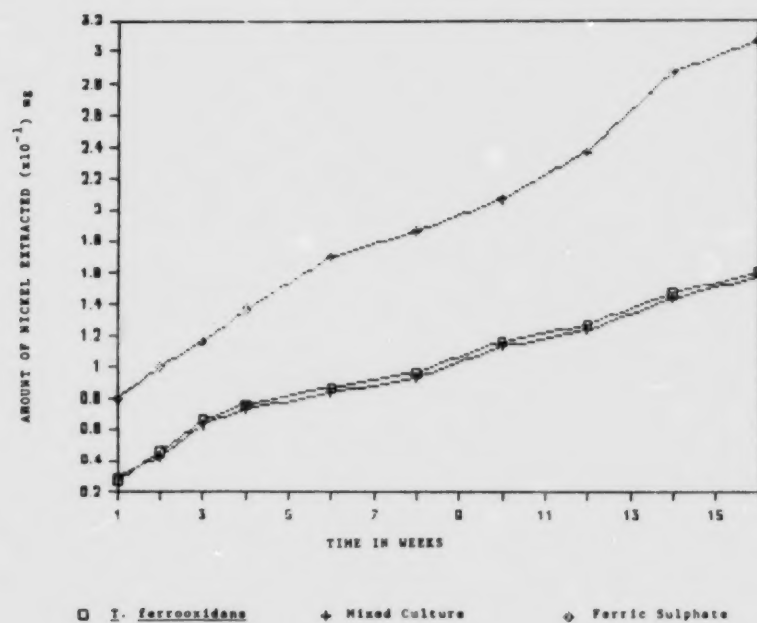


Fig. 4 Cumulative Extraction of Nickel from Complex Sulphide Ore Discs by Various Treatments

SESSION II

METAL RECOVERY AND ENVIRONMENT

SESSION II: PAPER 6

V.I. Lakshmanan, J. Christison, D.J. King
M. Tsezos and R.G.L. McCready

ABSTRACT

Uranium adsorption tests from Denison minewater adjusted to pH 4.0 were carried out. Effect of nutrients, age, and immobilization procedures on biomass production and uranium adsorption were tested.

The preliminary results have indicated uranium adsorption at up to 150 mg/g dried biomass, Medium TM as carbon source yielded 20-24 g dry wt/L *Penicillium* biomass within 18-60 hours. Preliminary tests show that the *Penicillium* biomass can be converted to immobilized form to provide uranium adsorption at up to 150 mg/g dry biomass. Further confirmatory lock cycle loading/elution/regeneration column test studies are suggested.

SESSION II: PRÉSENTATION 6

Optimisation de l'adsorption fongique de l'uranium
et immobilisation cellulaire

RÉSUMÉ

Des essais d'adsorption d'uranium ont été effectués sur des eaux de la mine Denison dont le pH avait été réglé à 4,0. L'effet des nutriments, de l'âge et des procédures d'immobilisation sur la production de biomasse et l'adsorption d'uranium a été vérifié.

Les résultats préliminaires ont révélé que l'adsorption d'uranium à un taux atteignant 150 mg/g de biomasse séchée, dans un milieu TM comme source de carbone, produisait de 20 à 24 g en poids sec/L de biomasse de Penicillium en 18 à 60 heures. Les essais préliminaires indiquent que la biomasse de Penicillium peut être convertie sous forme immobilisée pour produire un taux d'adsorption d'uranium atteignant 150 mg/g de biomasse sèche. On recommande de confirmer ces résultats par des études expérimentales de chargement/élution/régénération en circuit fermé sur colonne.

OPTIMIZATION OF FUNGAL ADSORPTION OF URANIUM AND CELL IMMOBILIZATION

1. INTRODUCTION

Denison Mines in co-operation with the Department of Energy, Mines and Resources, is recovering uranium using bacterial leaching technique. The volume of leachate obtained under such conditions is high with low uranium tenor. The cost of pumping such high volume leach liquor is high. The Department of Energy, Mines and Resources is at present evaluating the technical and economic potential of recovering the contained uranium in minewater underground and hence reduce the cost of pumping and recovering uranium on surface.

The potential of micro-organisms to recover uranium from minewater was earlier investigated under DSS contract (15 SQ. 23440-5-9181) by Acres Davy McKee Engineering Ltd. The adsorption rates, capacities, desorption characteristics, the effect of the pH and other cations on loading were studied. Amongst the treated micro-organisms, Penicillium provided higher adsorption and desorption characteristics. However, uranium adsorption by Penicillium was only about 25 mg/g of dry penicillium. Further the biomass as produced was found to be physically less stable, and hydrodynamically difficult to handle for column operations.

In the present paper preliminary results obtained to investigate nutrient requirement to increase the uranium loading capacity to approximately 200 mg/g and evaluate the potential to immobilize the biomass and use for uranium adsorption are discussed. The test work was carried out under a contract awarded by Department of Energy, Mines and Resources (DSS Contract No. 23440-6-9052101-SQ).

2. EXPERIMENTAL

2.1 Culture

The culture used in these tests was the acidophilic Penicillium SP isolated by Dr. R.G.L. McCready, CANMET, from the bacterial leach stopes at Denison Mines, Elliot Lake.

Stock cultures were maintained on agar slants (0.1 M strength nutrient broth + 1% (w/w) glucose, pH 2-3) at 4°C.

2.2 Basal Medium for Growth Studies

In order to undertake tests to assess the growth of the *Penicillium* Sp on different carbon sources, a basal medium was prepared consisting of:

1. Ammonium Nitrate	20 g/L
2. Potassium Dihydrogen Phosphate	11 g/L
3. Magnesium Sulphate (7H ₂ O)	0.25 g/L
4. Zinc Sulphate (7H ₂ O)	0.03 g/L
5. Ferrous Sulphate (7H ₂ O)	0.05 g/L
6. Manganous Sulphate (7H ₂ O)	0.02 g/L
7. Ammonium Molybdate 47H ₂ O)	0.005 g/L

The medium was prepared as follows:

Solution A

The ammonium nitrate, potassium dihydrogen phosphate and magnesium sulphate were dissolved in distilled water and made up to 750 ml. The pH was adjusted to 5.0 with sulphuric acid prior to autoclaving at 16 psi for 25 minutes.

Solution B

The 100x concentrated solution of zinc sulphate, manganous sulphate and ammonium molybdate was prepared with distilled water, and the pH was adjusted to 5.0 with sulphuric acid prior to sterilization at 16 psi for 25 minutes.

Solution C

A 100x concentrated solution of ferrous sulphate was prepared in distilled water, pH 5.0, and sterilized by filtration (0.22 μ m) immediately before use.

The basal salts medium was prepared for tests by aseptically mixing the appropriate volumes of solutions A, B & C.

The carbon source being evaluated was dissolved in the appropriate volume of water and sterilized separately at 16 psi for 20 minutes. It was added to the basal salts solution, and if necessary sterile distilled water was added to appropriate volume. During testwork cultures under shake flask and stirred tank fermenter conditions were prepared as required.

Harvesting Procedures

The preferred procedure involved collection of the biomass by filtration through cheese cloth, washing of biomass with warm water until the eluate was relatively free of colour, suspension of the biomass in warm tapwater for 30 minutes, refiltration on to cheese cloth, repetition of the warm water wash, inactivation of biomass by autoclaving and freeze drying.

Uranium Adsorption Tests

The minewater (pH 2.05) as received from Denison Mines was adjusted to pH 4.0 with caustic filtered and used for stirred tank bioadsorption tests. Multi-elemental analysis of pH adjusted minewater is shown in Table 1.

3. RESULTS AND DISCUSSION

During the test program, optimum growth medium, effect of aging on uranium biosorption, and preliminary investigation on immobilization and adsorption of uranium with immobilized biomass were studied.

3.1 Selection of Optimum Growth Medium

Factors that have significance under optimum conditions include biomass yield and cost of nutrients. During the test program, a refractory carbon source as supplied by

Prof. M. Tsezos (Medium M), hexadecane, potato starch, lactose and malto dextrine were tested as carbon source.

3.1.1 Selection of Culture Conditions

Preliminary cultures in shake flasks using Medium TM showed that apparent decline in the yield of biomass per unit volume of culture suspension as the culture aged (Fig. 1). It was also observed that medium strength influenced the type of mycelial growth. Thus in more dilute media (Media TM/4, TM/2), the biomass consisted of larger pellets. Since fibrous growth was preferred for immobilization procedures, this pelletized growth was not desired.

3.1.2 Comparison of Carbon Sources

Based upon the preliminary shake flash tests, it was apparent that high yields of biomass could be obtained with Medium TM and that the maximum biomass yield per unit volume of culture was proportional to the carbon concentration of the respective strengths of medium (Fig. 1). Thus other nutrients were not limiting.

In an earlier study, biomass cultures in medium containing predominantly glucose as a carbon source had lower uranium adsorption capacity. It was presumed that this may be due to excess production of reserve storage compounds in the presence of an easily metabolized energy source. Accordingly refractory carbon sources (Medium TM) which will slowly be utilized by organisms were studied. As discussed earlier industrial waste carbon sources such as lactose, malto dextrine, potato starch, hexadecane and cheese whey were also studied. When industrial waste products were used as carbon source, foaming was a major problem. Good yields of biomass were obtained but the uranium adsorption capacities proved to be poorer than biomass cultured on Medium TM (Table 2). Accordingly Medium TM was chosen for further studies.

3.1.3 Medium TM

The analysis of TM concentrate is as follows:

Total Solids	28% (w/v)
Total phosphorus	1.1% (w/v)
Magnesium	0.5% (w/v)
Total Kjeldal Nitrogen	11% (w/v)
Total Organic Carbon	11% (w/v)

Initial tests carried out using medium prepared by dilution of sterile concentrate shows the development of contaminant organisms prior to the initiation of the fungal growth. This was possibly due to the production of toxic degradation products or to the destruction of essential nutrients co-factors in the medium. Hence non-sterile concentrate Medium TM has to be diluted prior to sterilization in-situ fermenters. Inoculated cultures showed immediate and rapid growth with growth completed within 40-48 hours (Figs. 2-3). The medium prepared in this fashion was much lighter in colour and the growth curve was confirmed in a later test (Fig. 4).

3.1.4 Effect of Age on Uranium Biosorption Capacity

Comparison of uranium loadings obtained under equivalent test conditions (0.1 g dry biomass/200 mL minewater) with biomass samples at intervals along the stationary phase of growth indicated that the age of the biomass had only a slight impact on the observed uranium loading (Table 3).

3.1.5 Production of Biomass for Immobilization

During the growth studies, it was noted that the start of the stationary phase of growth was associated with a sharp rise in the pH of the medium. This inflection in the pH of the medium was used to indicate when a culture should be harvested. Biomass samples collected under such test

conditions yielded biosorption capacities up to 260 mg/g and 320 mg/g. However, these loadings were obtained at high liquid/solid ratios in stirred tank tests and need confirmation under column loading conditions.

3.1.6 Assessment of Immobilized/Pelletized Biomasses

Penicillium biomass obtained using Medium TM carbon source was immobilized using Tsezos pelletization and ORF sol-gel procedures. The biosorption results are shown in Table 4. The stirred tank test results show loading at up to 150 mg/g and will need to be confirmed under lock cycle column loading, elution, and regeneration conditions.

TABLES

TABLE 1

MULTI ELEMENT ANALYSIS OF pH 4 MINE WATER

Element	Sample N ppm	Sample O ppm
Co	4.8	4.2
Zn	18	12
Cd	0.05	<0.05
B	0.76	0.74
Bi	<0.2	<0.2
P	<0.5	<0.5
Be	0.02	0.02
Si	25	23
Fe	3.0	1.0
Mn	2.1	2.5
Ca	140	150
Mg	16	26
Cu	2.6	1.9
Al	48	59
V	<0.02	<0.02
Mo	<0.05	<0.05
Pb	<0.1	<0.1
Ni	2.1	2.2
Cr	0.06	0.05
Na	2590	1470
U	150	153

TABLE 2

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS GROWN ON VARIOUS
CARBON SOURCES

Feed Solution: Mine Water, pH adj. to 4.0

Contact time - 24 hours

Test No.	Biomass	Age (1) of Bio- mass (h)	Biomass g	Mine water mL	Feed U ppm	Solution U mg	Final U ppm	Solution U mg	Mg U loaded	Mg U/g Biomass
87-51	P2-12 (2)	159	0.014	200	120	24	111	22.2	1.8	129
87-52	P3-13 (3)	210	0.01	200	120	24	112	22.4	1.6	160
87-61	P4-11 (4)	270	0.1	200	51	10.2	6.3	1.3	8.9	89
87-62	P5 (5)	n/a	0.1	200	51	10.2	39	7.8	2.4	24
87-63	P6 (6)	200	0.1	200	51	10.2	13	2.6	7.6	76
87-64	PE7-1 (7)	n/a	0.1	200	51	10.2	18	3.6	6.6	66
87-65	PE7-2 (8)	n/a	0.1	200	51	10.2	30	6.0	4.2	42

1) See footnote - Table 2

2) Medium TM

3) Medium TM

4) Medium TM/4

5) Carbon source - hexdecane

6) Carbon source - cheese whey

7) Carbon source - potato starch

8) Carbon source - lactose

TABLE 3

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS-EXPERIMENT P8 (MEDIUM TM/2)
AND P9 (MEDIUM TM/1.5)

Feed Solution: Mine Water, pH adj. to 4.0

Contact time - 24 hours

Test No.	Biomass	Age (1) of Bio- mass (h)	Biomass g	Mine water mL	Feed U ppm	Solution U mg	Final U ppm	Solution U mg	Mg U loaded	Mg U/g Biomass
87-76	P8-12A	129	0.1	200	150	30	131	26.2	3.8	38
87-77	" "	129	0.1	400	150	60	133	53.2	6.8	68
87-78	" "	129	0.1	200	153	30.6	125	25.0	5.6	56
87-79	" "	129	0.1	400	153	61.2	133	53.2	8.0	80
87-80	" "	129	0.1	200	150	30	130	26.0	4.0	40
87-81	P8-15A	239	0.1	200	150	30	124	24.8	5.2	52
87-82	P9-11A	85	0.1	200	150	30	130	26.0	4.0	40
87-83	P9-13A	180	0.1	200	150	30	122	24.4	5.6	56
87-84	P8-Final	1385	0.1	200	177	35.4	171	34.2	1.2	12
87-85	P9-Final	1327	0.1	200	177	35.4	167	33.4	2.0	20

(1) See footnote - Table 2

TABLE 4

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS-P11 IMMOBILIZED/
PELLETIZED BY ORF SOL GEL PROCEDURE

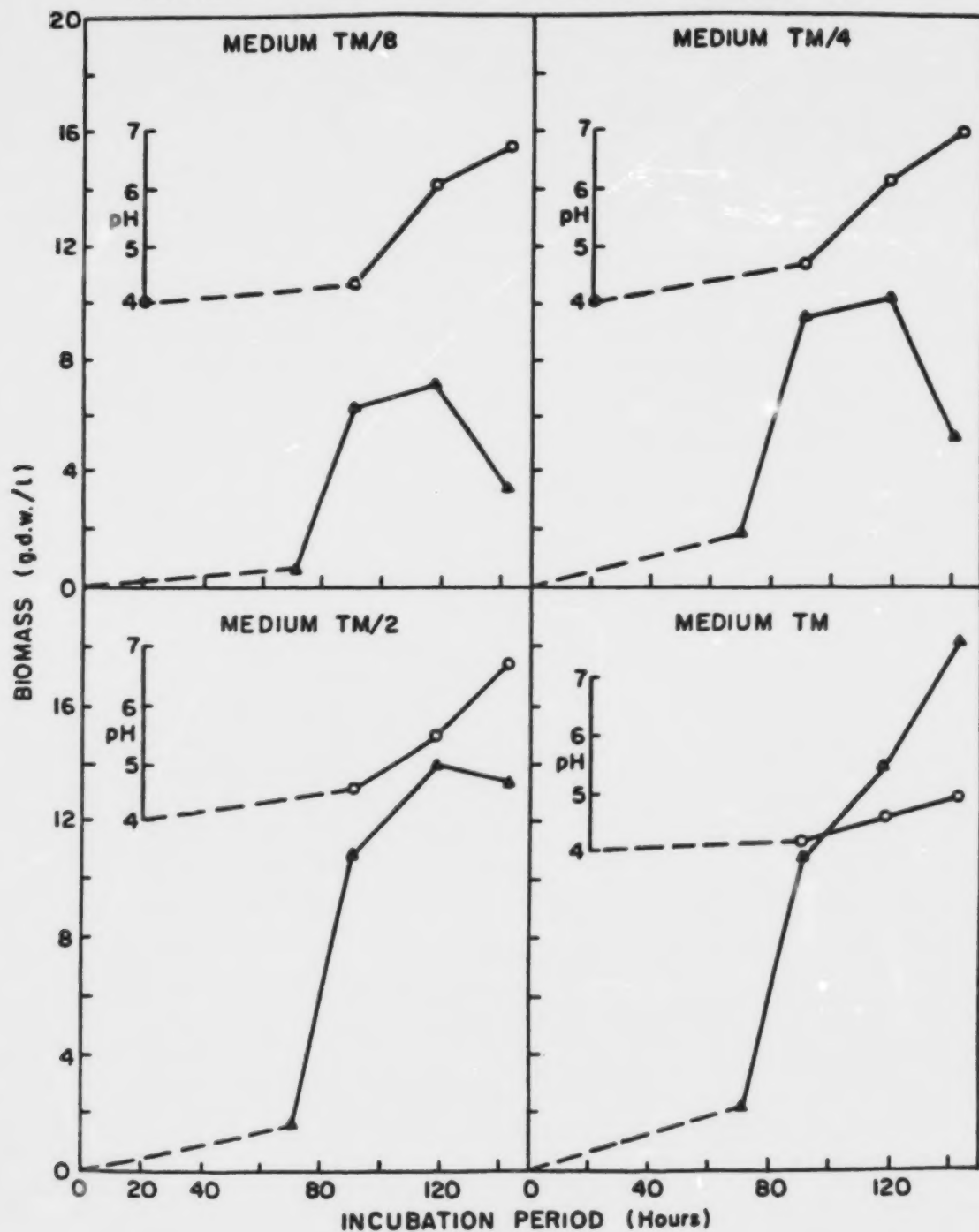
Feed Solution: Mine Water, pH adj. to 4.0

Contact time - 24 hours

Test No.	Biomass g	Biomass water	Mine U ppm mL	Feed U mg	Solution U ppm	Final U mg	Solution loaded	Mg U Pellets	Mg U/g
87-106	Wet	0.02	400	166	66.4	151	60.4	6.0	300
87-107	"	0.1	400	166	66.4	142	56.8	9.6	96
87-108	"	0.2	400	166	66.4	119	47.6	18.8	94
87-109	Dry	0.02	400	166	66.4	146	58.4	8.0	400
87-110	"	0.02	400	166	66.4	142	56.8	9.6	480
87-111	"	0.04	400	166	66.4	141	56.4	10.0	167
87-112	"	0.06	400	166	66.4	137	54.8	11.6	116
87-113	"	0.1	400	166	66.4	137	54.8	11.6	116
87-114	"	0.2	400	166	66.4	133	53.2	13.2	66

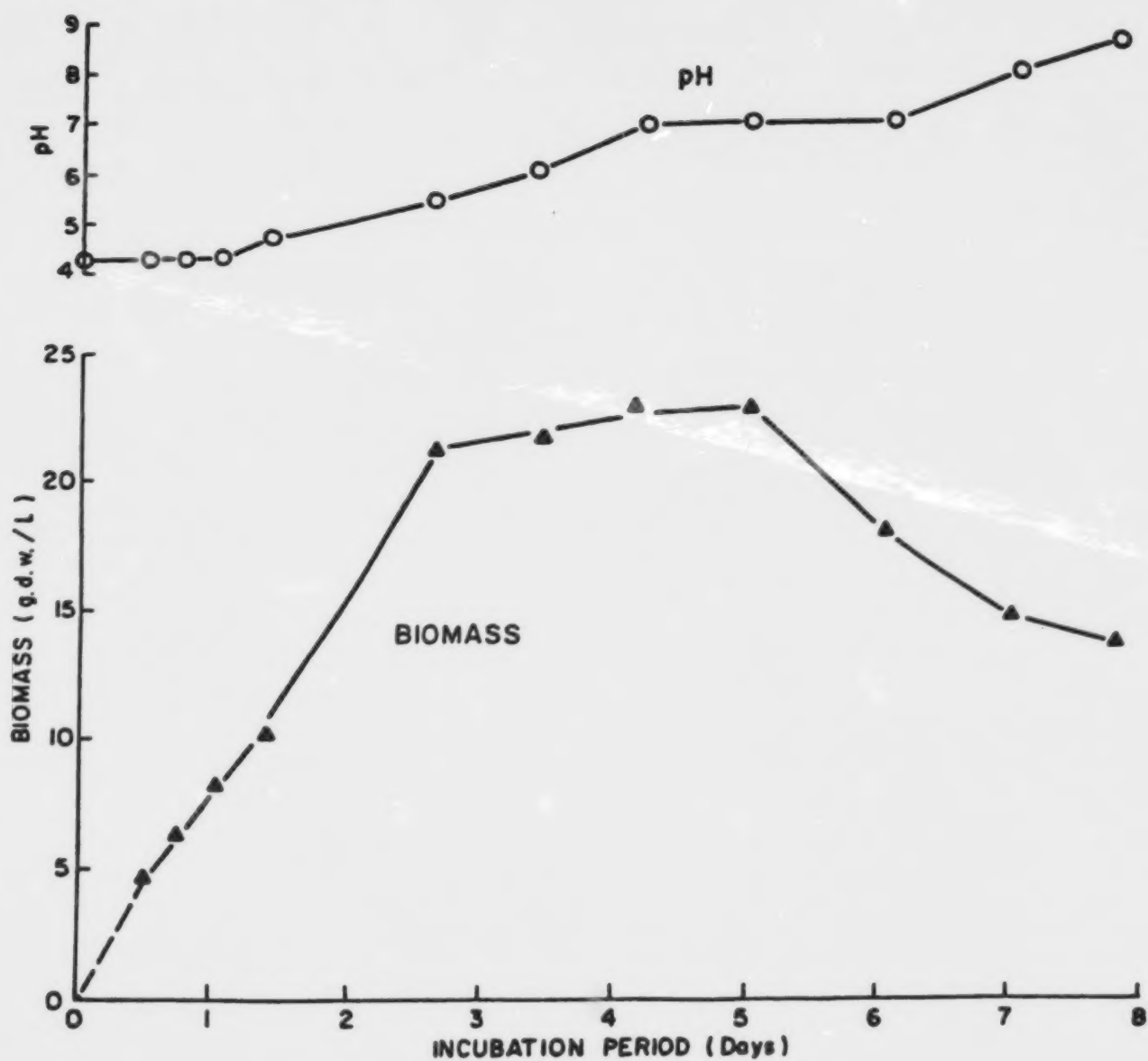
FIGURES

FIGURE 1 SHAKE FLASK CULTURES - MEDIUM TM



KEY: ▲ BIOMASS (g.d.w./l)
○ pH

FIGURE 2 GROWTH CURVE OF *PENICILLIUM* SP. IN MEDIUM TM
STERILIZED AFTER DILUTION OF CONCENTRATE
CULTURE P13



GROWTH CURVE OF PENICILLIUM SP. IN MEDIUM TM
- LARGE SCALE PRODUCTION OF BIOMASS
pH CURVE CULTURE P12

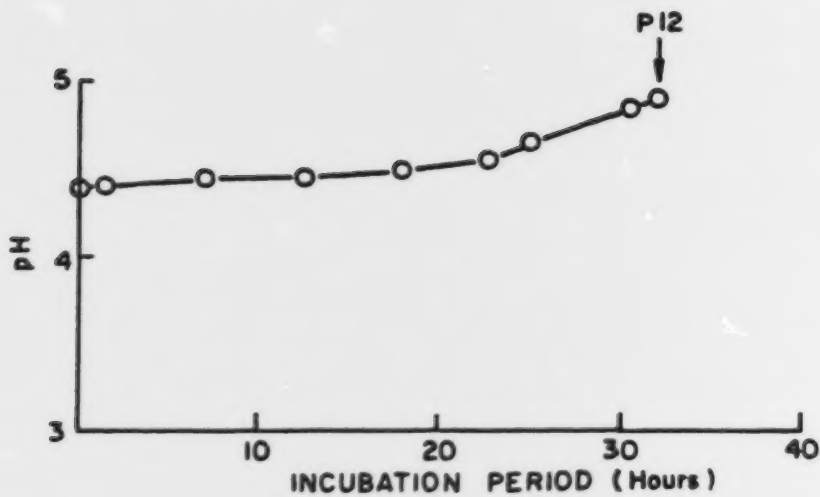
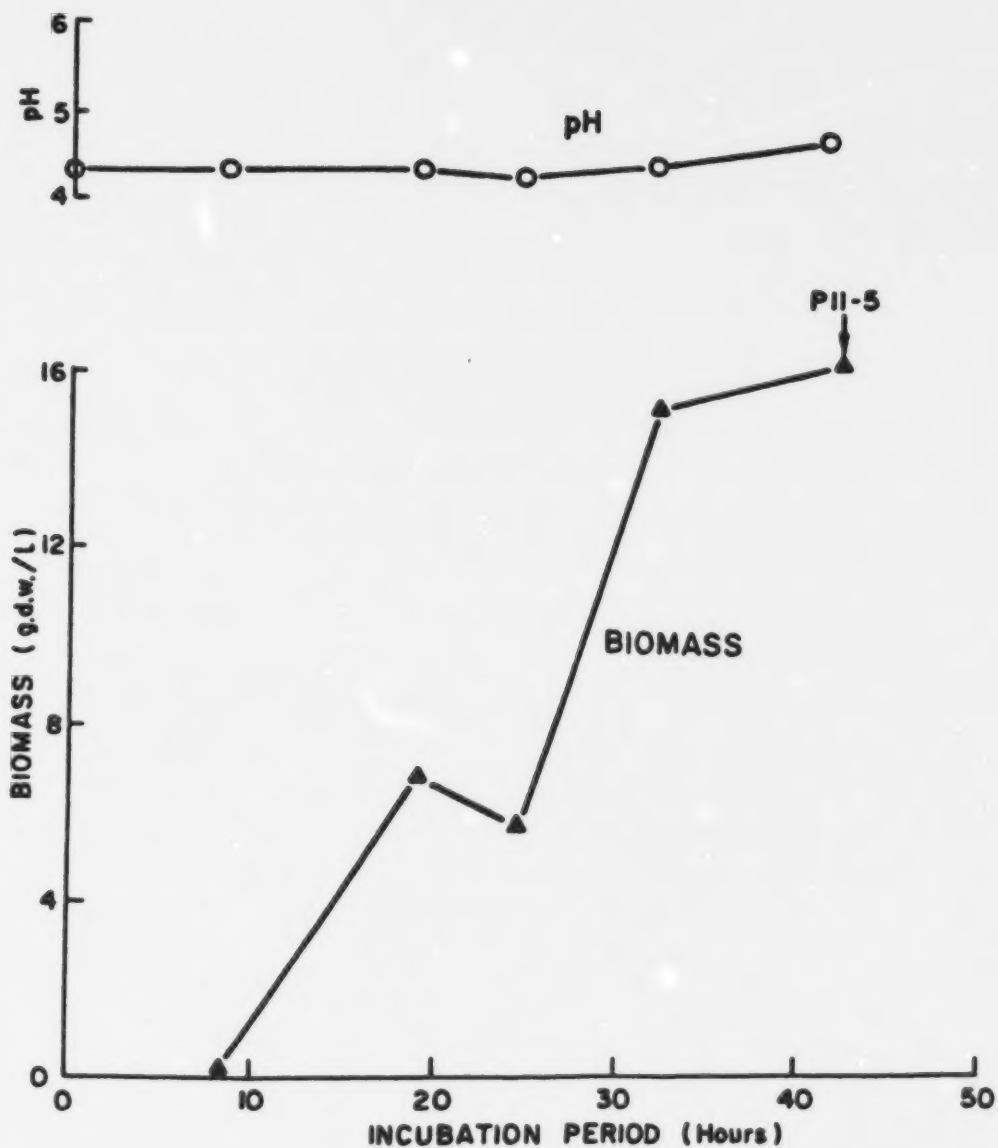


FIG. 4 GROWTH CURVE OF *PENICILLIUM* SP IN MEDIUM TM
- LARGE SCALE PREPARATION OF BIOMASS.
CULTURE PII



SESSION II: PAPER 7

R.G.L. McCready and J. Salley*

T.G. Smith**

ABSTRACT

Acidic smelter effluents from base metal mines contain 20-100 ppm of oxidized selenium salts. Many species of microorganisms including Corynebacterium diptheriae, Neurospora crassa, Candida albicans, Salmonella heidelberg and Escherichia coli are capable of assimilating selenite and intracellularly reducing it to red amorphous selenium.

CMS Rotodisk in co-operation with CANMET recently pilot-planted an industrial biological process proposed by CANMET for the recovery of elemental selenium from the acidic smelter effluents of an operating base metal mine. The results of this pilot-plant study will be presented.

Keywords: assimilation, reduction, selenium, selenite

* Extractive Metallurgy Laboratory, CANMET, Energy, Mines and Resources Canada, Ottawa, Ontario, K1A 0G1.

** CMS Rotodisk Inc. Mississauga, Ontario.

SESSION II: PRÉSENTATION 7

R.G.L. McCready et J. Salley*

T.G. Smith**

RÉSUMÉ

Les effluents acides de fonderie provenant de mines de métaux communs contiennent 20 à 100 ppm de sels de sélénium oxydés. De nombreuses espèces de microorganismes, en particulier Corynebacterium diphtheriae, Neurospora crassa, Candida albicans, Salmonella heidelberg et Escherichia coli peuvent assimiler la sélénite et la réduire dans le milieu intracellulaire en sélénium rouge amorphe.

En collaboration avec le CANMET, la CMS Rotodisk a récemment fait l'essai en usine-pilote d'un procédé biologique industriel proposé par le CANMET pour récupérer le sélénium élémentaire des effluents acides d'une fonderie recevant le minerai d'une mine où l'on extrait actuellement des métaux communs. On présentera les résultats de cette étude réalisée en usine-pilote.

Mots-clés : assimilation, réduction, sélénium, sélénite

* Laboratoire de métallurgie extractive, CANMET, Énergie, Mines et Ressources Canada, Ottawa, Ontario, K1A 0G1

** CMS Rotodisk Inc. Mississauga, Ontario.

BIORECOVERY OF SELENIUM

INTRODUCTION

From the literature it is apparent that a variety of organisms can reduce elements of group 6A of the periodic table via their intracellular assimilatory sulfate reductive pathway. Elements such as selenium and tellurium are competitive inhibitors of this pathway and thus, organisms which can transport the oxy salts of these elements across the cytoplasmic membrane can reduce the salt to the elemental state intracellularly.

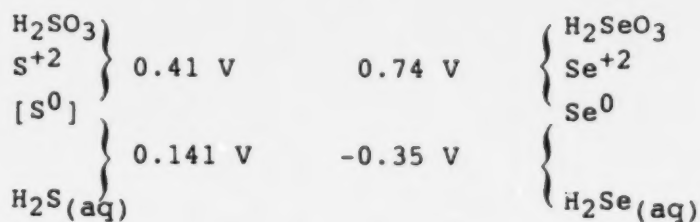
Selenite is metabolized by microorganisms in at least two different ways: for example, by the enzymatic utilization of SeO_3^{2-} in lieu of sulfur, presumably by converting SeO_3^{2-} to the divalent selenide (Se^{2-}), and forming therefore such products as selenomethionine and selenocysteine (Cowle & Cowen, 1957; Tuve & Williams, 1961); competitive inhibition by these seleno-amino acids could account for the toxicity of SeO_3^{2-} to many species. In addition some species reduce SeO_3^{2-} to an insoluble product which is deposited intracellularly. The intracellular deposition of such precipitates from SeO_3^{2-} has been shown in Corynebacterium diphtheriae (Levine, 1925), Neurospora crassa (Zolotar, 1953), and Candida albicans (Falcone & Nickerson, 1963)

The use of SeO_3^{2-} as a selective agent in enrichment media for the isolation of Salmonella from natural sources was first recommended by Guth (1916), although general acceptance was not forthcoming until the publication of the later work of Leifson (1936).

The mechanism of SeO_3^{2-} reduction by bacteria was studied as part of an investigation of biological reduction of group 6A elements (S, Se, Te). The tolerance of Salmonellae to SeO_3^{2-} is shown to involve an active process wherein selenite is converted to an insoluble form (Se^0) and the medium thus detoxified.

McCready et al., 1966 showed that the reduction of selenite occurred via two, two electron transfer steps;
 $\text{Se}^{4+} \text{ -----} \rightarrow \text{Se}^{2+} \text{ -----} \rightarrow \text{Se}^0$; and the reduction occurred at different rates depending on the organism used to mediate the reaction (see Table 1)

These authors concluded that since sulfur and selenium are both in group 6A of the periodic table and since they have similar chemical and physical properties, the reductive enzyme may be the same or similar in the two systems. S. heidelberg reduces Na_2SO_3 to H_2S . Both SO_3 and SeO_3 pass through the same valence states on reduction; however, the difference in the end product valence state may be due to the great differences in the oxidization-reduction potentials of the two systems. The following represents the oxidation-reduction potentials of the two systems.



In the sequential reduction of SO_3^{2-} elemental S^0 is not released as is Se^0 , but may remain organically bound and further reduced to S^{2-} .

Using this background information a process design based on a rotary biological contractor was developed for the removal of selenium salts from mine effluents and was recently pilot tested by CMS Rotodisk in Mississauga.

LABORATORY STUDIES

Selenite reducing organisms were grown in Trypticase Soy Broth (Baltimore Biological Co. Ltd.) containing 0.1% w/v SeO_3^{2-} (456 ppm Se) at 37°C. The growth, changes in pH, and deposition of intracellular selenium granules were followed over a 32 hour period. The organisms were capable of reducing selenite to elemental selenium over the pH range of 6.0 to 8.5 with an optimal rate of reduction at pH 7.0 (See Fig. 1-4).

Simultaneously, the cultures were examined and photographed under phase contract microscope at various timed intervals. Granule deposition was observed from 4 to 12 hours after inoculation. When the selenite concentration had been substantially reduced, normal logarithmic growth of the cultures was observed.

PILOT-PLANT STUDIES

Under the NRC-IRAP program CMS Rotodisk Inc. conducted a pilot-plant study for the removal of selenium from a base metal smelter weak acid effluent. A laboratory scale rotary biological contractor with 100 sq. ft. of disk surface and a 30 L capacity was used for the study (Fig. 6).

The weak acid effluent which was neutralized to pH 7.0 was combined with the microbial inoculum and a nutrient solution as an input stream to the rotary biological contractor (RBC). Tests were conducted to optimize parameters such as C:Se ratio, flow rate and retention time.

Using the RBC > 97% of the selenium in the weak acid effluent is removed with a retention time of 4 hours. The selenium laden biomass that sloughs off the disks of the RBC is recovered from the RBC effluent by centrifugation.

As the selenium granules are within the cytoplasm of the bacterial cells, the cell walls and membranes must be ruptured in order to recover the metal.

CANMET is currently developing a process for the recovery of the selenium granules from the biomass. Tests are being conducted on various procedures to rupture cell walls and membranes including methanol:KOH extraction, and the use of lytic enzymes such as lipase and protease. To date, the recovered selenium has been about 90% purity with 0.02% sulphur, traces of potassium and the balance is presumed to be cellular carbohydrates or protein (see Table II). Tests are being conducted to remove the contaminating materials while attempting to keep the number of steps and the cost of the process to a minimum.

From the pilot-plant study and the selenium recovery project it has been estimated that the capital cost for treating 1100 gallons per minute of effluent at an average selenium content of 40 ppm would be about $\$3.4 \times 10^6$ with an annual operating cost of \$90,000. The potential return is $\$2 \times 10^6$ worth of selenium per year at the current market price of \$6 per pound.

REFERENCES

1. Burton, G.A., Giddings, T.H., Debrine, P. and Fall, R. 1987. High incidence of selenite-resistant bacteria from a site polluted with selenium, Appl. and Environ. Microbiol. 53;185-188.
2. Cowle, D.B. and Cohen, G.N. 1957. Biosynthesis by *Escherichia coli* of active altered proteins containing selenium instead of sulfur. Biochem. Biophys. Acta, 26, 252-261.
3. Falcone, G. and Nickerson, W.J. 1963. Reduction of selenite by intact yeast cells and cell free preparations. J. Bacteriol. 85, 763-771.
4. Guth, F. 1916. Selennährböden für die elektive Züchtung von Typhusbacillen. Zentra. Bakteriolog. I, Abt. 77, 487-496.
5. Leifson, E. 1936. New selenite enrichment media for the isolation of typhoid and para-typhoid (*Salmonella*) bacilli. Am. J. Hyg. 24, 423-432.
6. Levine, V.E. 1925. The reducing properties of microorganisms with special reference to selenium compounds. J. Bacteriol. 10, 217-263.
7. McCready, R.G.L., Campbell, J.W. and Payne, J.I. 1966. Selenite reduction by *Salmonella heidelberg*. Can. J. Microbiol. 12;703-714.
8. Tucker, F.L. Walper, J.F., Appleman, M.D. and Donahue, J. 1962. Complete reduction of tellurite to pure tellurium metal by microorganisms. J. Bacteriol. 83, 1313-1314.

9. Tuve, T. and Williams, H.H. 1961. Metabolism of selenium by Escherichia coli. J. Biol. Chem. 236, 597-601.
10. Zolokar, M. 1953. Reduction of selenite by Neurospora, Arch. Biochem. Biophys. 44, 330-337.

TABLES

Table I. The percentage of the population containing selenium granules during incubation in 0.1% selenite broth

Time of incubation (hours)	<u>E. coli</u>	<u>S. heidelberg</u>
4	33	83
6	52	90
8	79	95
10	91	97
12	94	95
27	68	10

Table II. Comparison of the X-ray diffraction analysis of the selenium from S. heidelberg with a selenium standard

Se° (A.S.T.M.)*		Se° (experimental)	
Diffraction, A°	Relative intensity, %	Diffraction, A°	Relative intensity, %
3.01	100	3.0	100.0
3.78	53	3.75	52.0
2.07	35	2.07	31.0
1.998	21	1.99	20.7
1.766	21	1.766	20.7

* American Society for Testing materials Index Card No. 6-0362

FIGURES

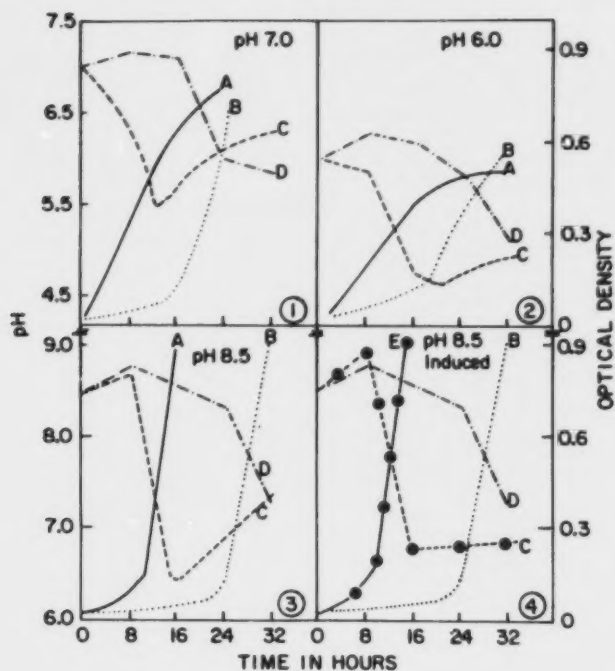


Fig. 1-4 Effect of SeO_3^{2-} on the growth of *Salmonella heidelberg*.

- Fig. 1 Initial pH 7.0. A = growth of control B = growth of selenite culture C = pH of control D = pH of selenite culture
- Fig. 2 Initial pH 6.0. A = growth of control B = growth of selenite culture C = pH of control D = pH of selenite culture
- Fig. 3 Initial pH 8.5. A = growth of control B = growth of selenite culture C = pH of control D = pH of selenite culture
- Fig. 4 Initial pH 8.5 inoculated with cells previously grown on selenite.
 E = growth of induced cells in selenite
 B = growth of non-induced cells in selenite
 C = pH of induced culture
 D = pH of non-induced culture

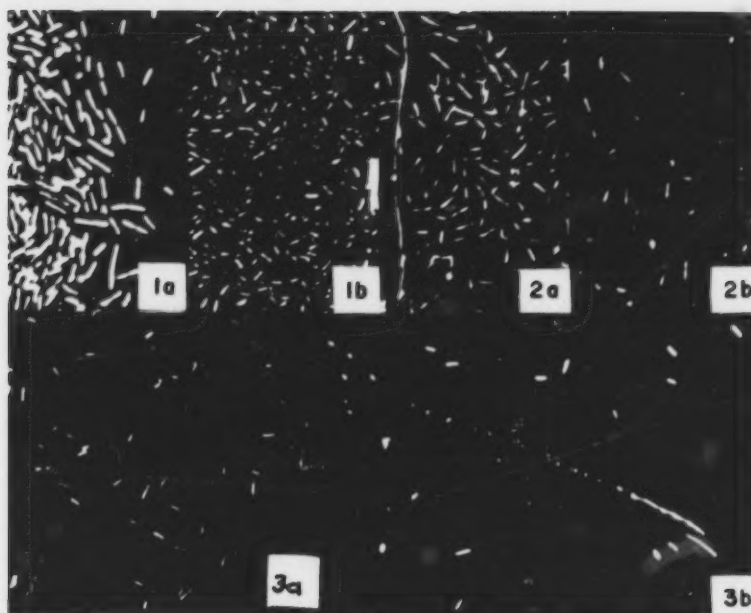


Fig. 5 Phase-contrast photomicrograph of Salmonella heidelberg 1200 X. 1a - 4 hr control; 1b - 4 hr SeO_3^{2-} culture; 2a - 8 hr control; 2b - 8 hr SeO_3^{2-} culture; 3a - 24 hr control; 3b - 24 hr SeO_3^{2-} culture.



Fig. 6 Laboratory scale rotary biological contractor.



SESSION II: PAPER 8

ABSTRACT

Bacterial and Chemical Leaching of Complex Sulphide Ores

V. Sanmugasunderam, W.D. Gould, R.G.L. McCready, S. Rajan,
M. Beaulne and P. Mainwaring.

CANMET

A major Canadian molybdenum and copper producer is occasionally plagued by the presence of a natural hydrocarbon (gilsonite) in the ore feed. A variety of mineral processing techniques have been tried in an effort to economically remove the gilsonite, but to no avail.

Witteck Development Inc., under contract to CANMET, studied the microbial alteration or degradation of gilsonite using known petroleum degrading organisms. Although this approach appeared promising, the time required was excessive and such a biological process would have been non-viable commercially.

From the Witteck studies it was obvious that the organisms were producing surfactants and emulsifiers which solubilized the viscous liquid hydrocarbons and allowed the graphitic carbon phase of the gilsonite to float free of the mineral sulfide concentrate. As a result of this observation, CANMET has initiated a program to assess commercially available surfactants and emulsifiers for the removal of the gilsonite from the concentrate. The results of these studies will be presented.

KEYWORDS: gilsonite, molybdenum, emulsifiers, surfactants,
graphitic carbon

SESSION II: PRÉSENTATION 8

McCready et Salley

RÉSUMÉ

Un important producteur canadien de molybdène et de cuivre rencontre parfois des problèmes insurmontables causés par la présence d'un hydrocarbure naturel (gilsonite) dans le minerai brut. On a fait l'essai de diverses techniques de traitement des minéraux pour éliminer économiquement la gilsonite, mais sans succès.

La Witteck Development Inc., aux termes d'un contrat conclu avec le CANMET, a étudié l'altération ou la dégradation microbiennes de la gilsonite, au moyen d'organismes connus qui décomposent le pétrole. Cette méthode semblait prometteuse, mais le temps requis était excessif, et ce type de procédé biologique ne serait pas économiquement rentable.

Des études de Witteck, il apparaît que les organismes produisent des surfactants et émulsifiants qui solubilisent les hydrocarbures ayant le caractère d'un liquide visqueux, et permettent à la phase graphitique de la gilsonite de flotter et de se séparer ainsi du concentré minéral sulfuré. À la suite de cette observation, le CANMET a lancé un programme pour évaluer dans quelle mesure les surfactants et émulsifiants disponibles dans le commerce permettent d'éliminer la gilsonite du concentré. On présentera les résultats de ces études.

MOTS-CLÉS : gilsonite, molybdène, émulsifiants, surfactants, graphite (graphitique).

PROGRESS ON EMULSIFICATION AND FLOTATION AS A MEANS OF UPGRADING A Cu/Mo CONCENTRATE

INTRODUCTION

The Cu/Mo concentrate produced from gilsonite contaminated ore contains 22-25% carbon. The gilsonite in the concentrate is about 50% viscous long-chain aliphatic hydrocarbon and 50% graphitic carbon (Table I). To date, metallurgical processing techniques have resulted in a maximum of 80% carbon rejection with quite significant molybdenum losses.

Table I

Elemental composition of the Cu/Mo concentrate

<u>Element</u>	<u>% w/w</u>
Mo	21.7-21.9
C	17.4-18.0
Fe	7.4-10.4
Cu	2.7-3.3
Gangue	50.8-46.4

Many genera of bacteria, including *Pseudomonas*, *Arthobacter*, *Achromobacter* and *Citrobacter* are known to degrade aliphatic hydrocarbons under aerobic conditions. As the viscous hydrocarbon portion of the gilsonite appeared to be acting as a bridge or cement between the mineral sulfides and the graphitic carbon in the concentrate, it was postulated that if the aliphatic hydrocarbons could be emulsified or degraded, then the refractory graphitic carbon could be separated from the mineral sulfides by conventional metallurgical processes.

SYNOPSIS OF THE WITTECK CONTRACT

Hydrocarbon degrading bacteria were isolated from samples obtained from the Petro Canada Clarkson Refinery and from the Esso Refinery in Sarnia. Initial shake flask studies on the contaminated concentrate were not too promising as the rates of hydrocarbon degradation were slow. In order to determine whether or not a component of the gilsonite was inhibitory or whether Cu, Mo, or other trace metals of the ore were inhibitory a new research approach was required. Gilsonite can be quantitatively removed from the concentrate by benzene extraction. The extracted viscous hydrocarbon tar was dissolved in a minimal volume of benzene and added to 250g of acid-washed Ottawa Sand. The benzene was evaporated, and a synthetic "tar-sand", free of metal sulfides, was available as a test substrate for the hydrocarbon-degrading organisms.

Using this technique the organisms were capable of degrading about 30% of the "tar-like" hydrocarbon coating on the sand in a fifteen day period. (See fig. 1-3) Similar shake-flask studies were completed using mixed cultures of bacteria with the gilsonite-contaminated Cu/Mo concentrate.

A pilot-scale bacterial treatment of 78Kg of Cu/Mo concentrate was run for an 8 day period. Following the bacterial pretreatment the slurry at 12.5% solids was cycloned to separate the carbon from the molybdenum concentrate. The best previous cycloning results obtained by the mine were 80% carbon rejected at 12.5% solids. Cycloning of the bacterially treated concentrate under the same conditions resulted in 90% carbon rejection but with significantly higher molybdenum losses than those reported by the mine in their cycloning studies.

In Phase II of the contract, Witteck switched from a batch-type reactor to a continuous-flow reactor to determine whether or not a continuous input of inorganic nutrients would increase the rate of hydrocarbon degradation. A 20% slurry of concentrate (187 L) was treated with the hydrocarbon degrading organisms for 80 hours at 20°C. During treatment 1.83% of the carbon was lost via the decant overflow from the 16 L continuous-flow reactor. (see fig. 4 & 5)

The bacterially-treated concentrate was then subjected to a variety of physical separation techniques in an attempt to remove the carbonaceous material (see Table II).

Table II

Effect of physical separation techniques on carbon removal from the Mo Concentrate.

<u>Technique</u>	<u>Sample</u>	<u>% Carbon Removal</u>	<u>% Mo loss</u>
Hydraulic	Bacterially Treated	0.95	0.66
Elution	Untreated	0.79	0.18
Heavy Liquid	Bacterially Treated	24	0.21
Separation	Untreated	18	0.14
Sp. gr. 1.27			
Flotation	Bacterially Treated	19.37	14.5
	Untreated	20.1	17.6
Cycloning*	Bacterially Treated	74.45	39.15
	Untreated	69.28	34.34

* Mo losses are believed high due to high slime content of the concentrate.

Heavy liquid separation gave the best carbon rejection with minimal molybdenum loss whereas flotation and cycloning resulted in excessive molybdenum losses due to the very fine particle size of the MoS_2 .

As 80 hours treatment resulted in contamination of the bacterial slurry with a predatory protozoa on several occasions and resulted in minimal improvement in carbon rejection; a new, chemical approach to the problem was initiated.

CANMET has surveyed the effect of treating the concentrate with various commercial surfactants. A slurry of concentrate (10% solids w/v) with 0.1% surfactant added was stirred at 200 rpm for 30 minutes. The slurry is allowed to settle for 30 minutes and the floating material is decanted, dried at 80°C, and the residue was weighed and analyzed for carbon and molybdenum content (Table III).

Table III

Carbon and Molybdenum rejection from 10 g of the mineral concentrate by surfactant treatment and flotation.

<u>Surfactant</u>	<u>Wt of float residue, g</u>	<u>%C</u>	<u>%Mo</u>
Alconox	0.80	33.6	3.1
Spic and Span	1.04	22.0	10.6
Decon 75	1.13	25.6	5.9
Triton X100	0.80	46.8	2.3
Liqui-Nox	0.57	40.9	3.4
Sodium dodecylsulfate	0.58	36.7	2.1
Tween 40	0.80	43.6	3.2
Tween 60	0.85	45.0	3.1
Tween 80	0.71	42.6	2.4

From this initial screening of the surfactants, Triton X-100 gave the maximum carbon rejection with the minimal molybdenum loss.

Another screening of surfactants in the Triton series was undertaken using the same experimental conditions as outlined above. These results are presented in Table IV.

Table IV

Effect of Triton series surfactants on carbon rejection from the Cu/Mo concentrate.

<u>Surfactant</u>	<u>Wt of residue (g)</u>	<u>%C</u>	<u>%Mo</u>
X-100	0.83	46.9	2.2
X-15	0.88	16.74	3.2
QS-15	1.06	40.77	-
CF-21	0.57	42.61	3.4
CF-54	0.45	39.9	5.1
X-151	0.50	42.0	6.3
W-30	0.24	13.7	3.8
X-45	0.28	39.3	3.9

Following discussions with the manufacturer of Triton X-100, CANMET is currently assessing combinations of the Triton X-100 and related surfactants with various emulsifiers which are compatible with these surfactants. Hopefully the combination of the surfactant and an emulsifier will be more effective than either reagent alone.

FIGURES



Fig. 1. Synthetic "Tar sand"



Figure 2. Synthetic "Tar-sand" in minimal salts medium prior to inoculation

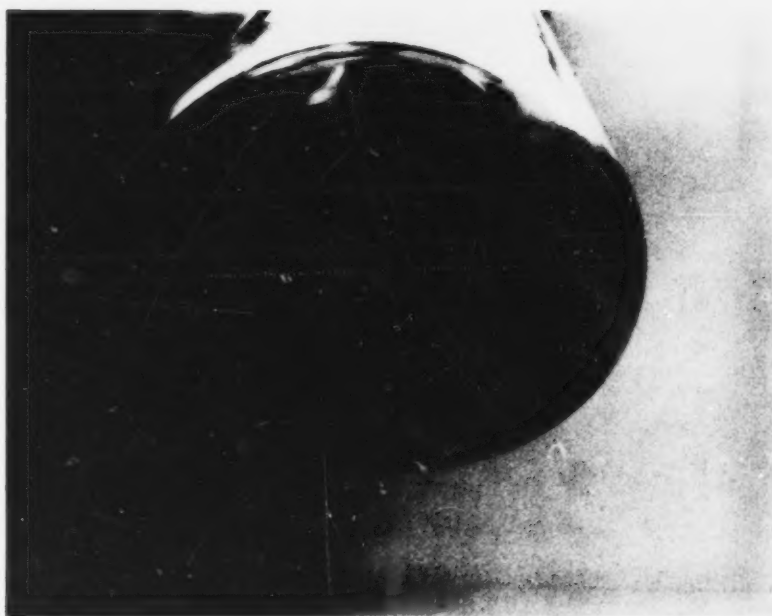


Fig. 3 Synthetic "tar sand" after bacterial treatment for 15 days

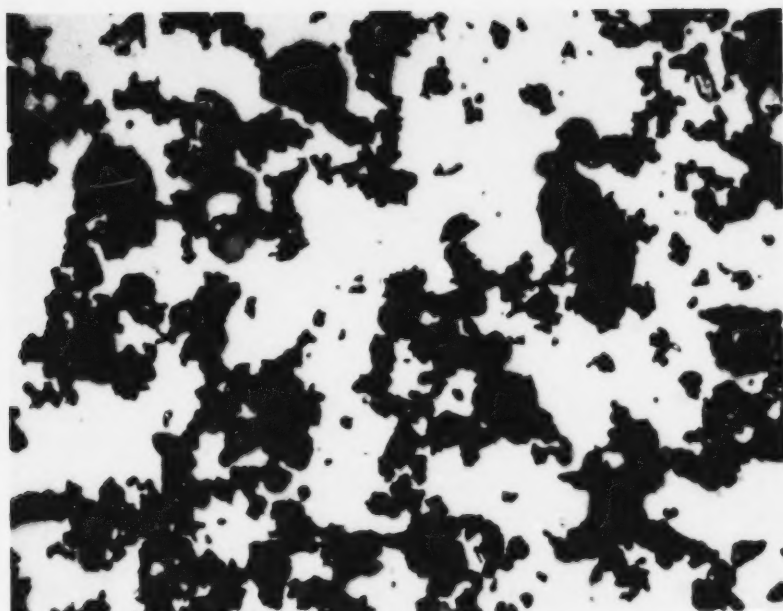


Fig. 4 Photomicrograph of untreated Cu/Mo concentrate

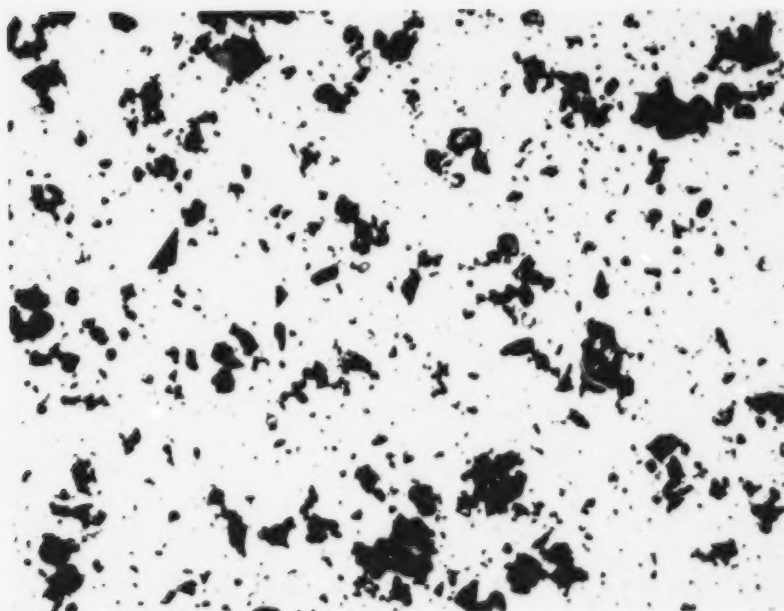


Fig. 5 Photomicrograph of the Cu/Mo concentrate after bacterial treatment

SESSION II: PAPER 9

M. Kalin
BOOJUM RESEARCH LIMITED
139 Amelia Street
Toronto, Ontario
M4X 1E6

This paper reports the development of biological methods which will lead to the curtailment and treatment of acid mine drainage from base metal operations. The ameliorative functions which cattails can perform are summarized. Cattails reduce the active layer in which acid is generated through their alteration of the biological activity in the rhizosphere. Cattails provide an organic layer on the tailings surfaces which intercepts precipitation, a large fraction of which is transpired by these plants. Furthermore, cattails provide nutrients as organic matter to sulphate-reducing bacteria, inducing metal precipitation.

In an effort to develop methods by which cattail populations may be established on these areas, experiments have been carried out on acidic (pH 2 to 3) and alkaline (pH 8 to 10) tailings surfaces over a 3 year period. Hydroponic, hand and mechanical transplant methods are discussed and the results obtained to date

are presented, together with observations on cattail seedling populations. The success of cattail population establishment in areas where no natural colonization occurs is a function of the methods chosen, as well as the chemical and physical conditions of the transplant locations.

SESSION II: PRÉSENTATION 9

DÉVELOPPEMENT DE MÉTHODES TECHNIQUES POUR PROTÉGER L'ENVIRONNEMENT EN RÉDUISANT LE DÉBIT DES EFFLUENTS MINIERs ACIDES

par

M. KALIN

BOOJUM RESEARCH LIMITED

139, rue Amélia

Toronto (Ontario)

M4X 1E6

RÉSUMÉ

Le présent document fait état du développement de méthodes biologiques permettant de réduire et de traiter les effluents miniers acides résultant du traitement des métaux communs. Un résumé des effets positifs des quenouilles est présenté. Celles-ci réduisent la couche active dans laquelle l'acide se forme en modifiant l'activité biologique de la rhizosphère. Les quenouilles forment une couverture organique à la surface des résidus qui intercepte les précipitations dont une grande partie est absorbée par ces plantes. De plus, les quenouilles constituent une source d'éléments nutritifs sous forme de matière organique pour les bactéries qui éliminent les sulfates qui provoquent les précipitations de substances métalliques.

Des expériences, échelonnées sur une période de trois ans, ont été effectuées en vue de développer des méthodes pour cultiver des populations de quenouilles dans ces régions. Ces expériences ont porté sur les surfaces recouvertes de résidus acides (pH 2 ou 3) et alcalins (pH 8 à 10). Le rapport fait état des méthodes de transplantation hydroponique, manuelle, et mécanique auxquelles on a eu recours, et comprend une étude des résultats obtenus à ce jour, de même que des remarques sur l'ensemencement de populations de quenouilles. La réussite des travaux effectués en vue d'établir des populations de quenouilles dans les régions dépourvues de ces plantes dépend de la méthode choisie de même que des conditions chimiques et physiques de l'emplacement sélectionné pour effectuer les transplantations.

PROGRESS IN THE DEVELOPMENT OF ECOLOGICAL ENGINEERING METHODS FOR CURTAILMENT OF ACID MINE DRAINAGE

INTRODUCTION

Wetlands perform several essential functions in ameliorating acid mine drainage. They produce a saturated cover of living organic material which provides anoxic conditions, i.e. curtailing oxygen penetration, thereby inhibiting or reducing microbial and chemical oxidation processes. The dead organic matter produced in the wetland serves a polishing function due to its capacity to absorb/adsorb metals, while the living biota contribute via direct biological uptake of metals. The cleansing capacity of wetlands however, is limited by the growth rates and the biological productivity of the wetland system.

The use of wetlands as passive biological polishing systems for coal acid mine drainage has recently received extensive attention (Barth, 1986; Nawrot, 1985; Pesavento, 1987). Chemical parameters which may limit application of these biological systems have been defined in terms of the concentrations of iron, manganese and pH, as well as flow rates. Wetland systems may not be applicable if the acid mine drainage carries iron in concentrations greater than 300 mg/l, manganese greater than 100 mg/l, and a pH less than 2.0, (Pesavento, 1984). However, pyritic waste material from base metal operations frequently result in chemical conditions with pH 2.5 to 4.0, with iron concentrations greater than 10,000 mg/l, and manganese concentrations greater than 1,000 (Errington and Ferguson, 1987; Paine 1987;)

Although the chemical and physical conditions of acid mine drainage from base metal operations limit the use of wetlands in many circumstances, the development of selected biological processes has been extensively investigated. Ecological Engineering methods are based on the wetland concept aimed to reduce acid mine drainage from base metal operations. However, these biological systems should be comprised of different groups of biota which are all indigenous to the waste material. These systems may perform specific functions in curtaining acid mine drainage from base metal waste material (Kalin and van Everdingen, 1987).

Ecological Engineering and Biological Polishing methods can be described as biological tools to prevent/reduce acid mine drainage, rather than as treatment systems in the engineering sense. One of their main applications is at the time of abandonment. Ecological Engineering at the time of close-out can achieve a walk-away condition where the design accommodates the oxidation rates of the acid generating waste material. The biological systems are applied in such a manner as to reduce or curtail the oxidation process in order that the growth rates and polishing capacities of the biota exceed the rates at which the acid is being generated, i.e. environmental degradation is prevented.

The indigenous species which are used in Ecological Engineering and Biological Polishing are aquatic and terrestrial mosses, two groups of algae(periphytic mats and rhizophytic meadows), as well as cattails *T. latifolia*. Cattails perform the following functions within the Ecological Engineering methods:

- (a) The root region reduces the active acid generating layer of the tailings mass (Kalin, 1984). Extensive cattail stands on tailings provide a layer which intercepts precipitation into the tailings, thereby reducing the major source of oxygen which drives the acid generation process (Kalin & van Everdingen, 1987; van Everdingen & Krouse, 1987). Wet meadows are reported to transpire 135% of the total precipitation (Larcher, 1980). Although cattail stands on tailings may not transpire as effectively as wet meadows in natural conditions, a reduction of precipitation available for infiltration into the tailings mass can be expected.
- (b) Organic matter produced by the aerial components (leaves, stems and fruits) of cattail stands further serve as an organic carbon source which is the starting point in supporting a synergistic microbial process of colonization including sulphate reducing bacteria. Their potential use in the treatment of acid mine drainage water has been described by Wakao et al (1979), but has not been developed for any practical application.

In light of these applications for cattails, the factors controlling the establishment, growth and expansion of naturally colonizing stands on the waste material have been addressed (Kalin, 1984; Kalin & Buggeln, 1986). Experiments have been carried out to develop methods which promote cattail stand growth. The results of these transplant experiments are presented along with observations on growth of seedling populations growing on active tailings areas are presented.

Description of experimental sites

The waste management areas on which the experiments are carried out are inactive, i.e. no longer associated with an operating mill. They are located in Central and Northern Ontario, near Sudbury and Red Lake, respectively. Both are acid generating waste areas resulting from the milling of base metals (copper and nickel, lead and zinc, respectively). Areas with extreme alkaline waste material (mine slimes and treatment ponds), as well as acid water bodies with high metal concentrations, have been selected as experimental sites.

Seedling populations germinating on mine slimes (Kalin, 1987) and on tailings in an operating waste management area, located in Timmins, have been investigated. The waste water consists of a combination of smelter and mine water discharge, as well as lead zinc tailings.

Transplant methods

HAND-TRANSPLANT: Mature cattails (vegetative and those bearing fruit) were excavated with a shovel and planted in areas void of cattails, either as single plants or in groups of the mature plants.

MECHANICAL TRANSPLANT: With the use of a front end loader, root/rhizome mats from a cattail population were transplanted onto shores of waste water bodies with no cattails. Old plants were marked with flagging tape to facilitate the counting of new shoots.

HYDROPONIC TRANSPLANT: Individual plants were planted in racks constructed out of waste wood, providing two test conditions: (i) roots/rhizomes embedded in sphagnum moss, or (ii) suspended in netting directly in the water.

Seedling establishment and growth

The growth of cattail seedling populations which had germinated naturally on mine slimes were examined by estimation of percentage seedling cover for two growing seasons. The effects of fertilization and mulching of quadrants upon survival of the populations was examined. Measurement of the height of seedling populations of various sized naturally colonizing active tailings areas were made in order to determine growth rates.

RESULTS

Transplanting of single mature cattail plants has been carried out in 1985, while in 1986, mature cattails in groups in of 3 transplanted. In Table 1 the results are summarized, giving the number of plants in the stands at the end of the growing season in 1987.

In area 4, single transplants were performed on a relatively dry mine slime section of the site. Two months after transplant, the number of plants (shoots) comprising the populations had more than doubled. However, after two growing seasons, the number of plants (shoots) did not increase further. However, this area received extensive new additions of mine slimes 10 to 20 cm. deep. Further, burial of the basal portions of the plants appears to inhibit new plant development, as new shoots from the rhizomes are unable to penetrate the material (Kalin & Buggeln, 1984). In areas 1, 2 and 3 (Table 1) different cattail source stands were transplanted from groups of 3 cattails into areas of set and drier mine slimes. After two months, the number of new shoots developed indicated a doubling of the populations in all areas. After over-wintering and one growing season, these populations had, in all three areas, nearly tripled. These results suggest that transplanting in groups of 3 plants produced stands with a better potential to increase in the next growing season.

In Table 2, the results from the transplant experiments under various conditions, including mine slimes, are summarized. The number of cattails originally transplanted and percent survival is given; the growth period covering one winter and one growing season for all methods.

Mill Pond has sandy, abiotic sediment. Algal blooms occurred at the edges during some months of the season. The important characteristics of the water are metal concentrations of 200 to 300 mg/l of zinc, 20 to 40 mg/l of copper and mild conditions of acid drainage with pH ranges of 4 to 6. Decant Pond is a settling pond on the tailings area, with very high concentrations of zinc and copper in the sediment (2 to 4 % and 0.4 to 1 %, respectively). The concentrations of these metals are considerably lower than Mill Pond water (1 to 3 mg/l zinc, 0.01 to 0.5 mg/l copper). The pH in Decant Pond ranges between 5.5 and 7.5 over the season.

A comparison of the different transplant techniques indicates, that the hand transplanting is the only viable method for Mill Pond. These hand-transplants were carried out at the edges of the pond initially in 1986. At the end of the growing season, based on the results obtained in the mine slimes (Table 1), transplants were carried out in groups of 3 mature plants, planting a total of 80 groups throughout the entire pond area. The pond was fertilized in the new year and 50% of the clumps

produced new shoots by May. However by the end of the growing season all shoots were dead. It is reasonable to suggest that metal concentrations are inhibiting growth in the centre of the pond. On the edges however, the run-off from the sides of the pond must ameliorate the conditions such, that survival is possible.

In Decant Pond, the growth of the mechanical transplants considerably exceeded that of hand transplanting methods (123% survival). This difference in growth was probably due to both the protection provided by transfer of the large root mass during mechanical transplant and the exposure of hand transplants on the short of Decant Pond, an area which did not support natural colonization of cattails. The hydroponic populations did survive, but one raft was destroyed after over-wintering. Eight plants protected by sphagnum moss around the roots survived and produced 23 new shoots.

The population fluctuations in the transplanted cattail stands are somewhat larger than those derived from non-transplanted (undisturbed) stands. Increases and decreases in natural populations in one meter square range from 11 to 69 % and 26 to 44 %, respectively, over one growing season. These population characteristics indicate that the hand transplants in Mill Pond and Decant Pond grow similar to natural populations. This is not the case for cattails on the mine slimes(Table 1) or

the mechanical and hydroponic transplants in Decant Pond, which had higher percentage survival.

Although transplanting mature cattails will lead to the propagation of new stands on these waste sites, clearly the most desirable method of establishing cattail stands would be by seed. In early spring the mine slimes were densely covered with cattail seedlings. Twenty quadrants were marked for observation. Mulching with a thin straw layer in order to protect the seedlings somewhat prolonged their survival. However, most seedlings perished during the summer months. In isolated, shallow depressions, a few seedlings matured and persisted. From laboratory experiments addressing seed germination, it was found that the critical phase after germination is establishment, confirming field observations (Kalin & Buggeln, 1986). The moisture regime is presently considered the key factor during the establishment phase.

In an active tailings area, where the pH is generally around 3.3, establishment of cattail seedling populations of various age groups occurred throughout the entire summer months. Growth rates were determined for these populations, based on height measurements. In Table 3, the number of plants, their height and the growth rate for the period between June and July is presented.

A comparison was made to determine the growth rate per day between mature cattails growing in approximately 10 to 20 cm of water (Table 3 'wet') and seedlings which were growing in the same moisture regime. These populations were compared to stands growing in drier conditions (referred to in Table 3 as 'moist'). In the moist condition, 3 height classes were differentiated, Height classes 1 and 2 being seedling populations, and Height class 3 being new shoots emerging at the edge of a mature cattail stand.

Low growth rates of 0.4 cm /day are associated with those mature cattails and seedling populations growing in the wet areas. The change in the water saturation to more moist areas resulted in increased growth rates. The growth rate of the new shoots along the moist edges of a mature cattail stand of the same height class are the same as those of all populations growing on moist tailings. Thus, the stage of the growth of the cattails, described here by height class alone, although at different root and rhizome developmental stages, do not appear to be related to the growth rates, but rather are affected by the degree of water saturation.

These results suggest that during establishment of cattail populations from seed, water saturation is essential during the establishment phase, but further successful growth requires a water level reduction. Thus, in order to establish populations from seed, control has to be exerted, at the correct time, over

the water regime in the area where cattails are to be established.

CONCLUSION

Although cattails are aggressive colonizers of waste sites, promotion of their growth in areas too harsh for natural colonization can be circumvented by transplanting mature plants. The success of transplanting is affected by the choice of the method and the conditions of the location. This is particularly important in waters with high metal concentrations. Hydroponic methods are not suited for highly metal-contaminated waters, but promising results have been obtained in less contaminated areas.

From the investigations which addressed the use of seedlings to establish cattail populations, it can be concluded that control of the water level has to be exerted in the area where cattails are to be established at the correct time during the establishment phase of the seedlings.

ACKNOWLEDGEMENTS

This work was supported by Inco Ltd. - Sudbury operations, BP-Selco, and Kidd Creek Mines Limited.

REFERENCES

- Barth, R.C. 1986. "Reclamation technology for tailings impoundments. Part I: Containment". **Mineral & Energy Resources: A Review of Developments** 29(1):1-25.
- Errington, J. and K. Ferguson, 1987. "Acid mine drainage in British Columbia - today and tomorrow". **Proc. Acid Mine Drainage Seminar/Workshop**, March 23-26, 1987, Halifax, Nova Scotia. pp. 67-88.
- Kalin, M. 1984. "Long-term ecological behaviour of abandoned uranium mill tailings. 2. Growth patterns of indigenous vegetation on terrestrial and semi-aquatic areas". **EPS report #3/HA/2**.
- Kalin, M. 1986. "Developing a technique for reclamation of high iron sulphur tailings by establishing a marshland. Phase I - Selection of suitable plant material and methods of establishment". **CANMET report**.
- Kalin, M. and R.G. Buggeln, 1986. "Cattail stand development on base metal tailings areas". **Proc. Land Rehabilitation: Policy, Planning Systems and Operational Programs**, June 3-6, 1986, University of British Columbia, Vancouver, B.C. pp. 261-276.
- Kalin, M. 1987. "Ecological engineering. Tests of concepts and assumptions on Levack. Year 2: Confirmation of concepts". **Report submitted to INCO Limited**.
- Kalin, M. and R.O. van Everdingen, 1987. "Ecological engineering: Biological and geochemical aspects, Phase I experiments". **Proc. Acid Mine Drainage Seminar/Workshop**, March 23-26, 1987, Halifax, Nova Scotia. pp. 565-590.
- Larcher, W. 1980. **Physiological Plant Ecology**. Springer-Verlag, New York. 303 pp.
- Nawrot, J.R. 1985. "Wetland development on coal mine slurry impoundments: principles, planning and practices". **Wetlands and Water Management on Mined Lands**. **Proc. of a Conference**, October 23-24, 1985, The Pennsylvania State University. pp. 161-172.
- Pesavento, B.G. 1984. "Factors to be considered when constructing wetlands for utilization as biomass filters to remove minerals". **Treatment of Mine Drainage by Wetlands**. **Proc.** August 13, 1984, The Pennsylvania State University. pp. 45-49.
- Paine, P.J. 1987. "Historic and geographic overview of acid mine

drainage". **Proc. Acid Mine Drainage Seminar/Workshop**, March 23-26, 1987, Halifax, Nova Scotia. pp. 1-46.

van Everdingen, R.O. and H.R. Krouse, 1987. "Interpretation of isotopic compositions of dissolved sulfate in discharges from sulfide-bearing mine tailings". **Abstract submitted to 1988 Mine Drainage and Reclamation Conference**, Pittsburgh, PA, April, 1988.

Wakao, N., T. Takahashi, Y. Sakurai and H. Shiota, 1979. "A treatment of acid mine water using sulfate-reducing bacteria". **J. Ferment. Technol.** Vol. 57(5): 445-452.

TABLES

Table 1: Growth of transplanted cattails in saturated and dry areas of alkaline mine slimes.

Transplant year	1985	1986	1986	1986
Transplant area	4	1	2	3
# Transplanted	40	39	88	79
Total # of healthy plants after 2 mos.	96	74	158	151
New shoots	-	49	82	88
Dead plants	-	0	12	16
Total August 1987	80	135	231	214

Table 2: Comparison of transplant methods in alkaline settling pond and acidic metal contaminated water body.

Transplant method	Acidic metal contaminated pond Mill Pond			Alkaline settling pond Decant Pond		
	Growth 1986	Growth 1987	Percent survival	Growth 1986	Growth 1987	Percent survival
Mechanical	137	42	30	211	261	123
Hand single plants	55	42	76	140	96	68
Hydroponic rafts	16	0	0	16	23	144

Table 3: Growth rates of different height class cattail populations

Description	# of plants	average height in cm		Growth rate/day
		June	July	cm/day
Mature moist	50	102	137	1.3
Mature wet	40	159	171	0.4
Mature edge moist	9	20	47	1.3
2nd class moist	40	73	105	1.2
1st class moist	40	40	72	1.2
Seedlings wet	50	11	23	0.4

SESSION III

POSTER SESSION

POSTER SESSION

ABSTRACT

I. Suzuki et al., University of Manitoba

We have grown three Thiobacillus thiooxidans strains (Tt = ATCC 8085, SM-6 and SM-7) and seven Thiobacillus ferrooxidans strains (Tf-1 = ATCC 13661, Tf-2 = ATCC 19859, SM-1, SM-3, SM-4, SM-5, and SM-8) on elemental sulfur and eight T. ferrooxidans strains (Tf-1, Tf-2, SM-1, SM-2, SM-3, SM-4, SM-5 and SM-8) on ferrous iron as the sole energy source. Cu and Zn tolerance of these strains was studied both during growth and with resting cell suspensions.

During growth on sulfur SM-4, Tt, SM-3, SM-6 and SM-7 were tolerant of 50 mM CuSO_4 ; SM-7, SM-6 and SM-3 were tolerant of 200 mM ZnSO_4 ; and all the strains were tolerant of 200 mM FeSO_4 .

The sulfur-oxidizing activity of SM-3, SM-4, SM-6 and SM-7 was resistant to inhibition by 167 mM CuSO_4 and 417 mM ZnSO_4 .

Growth on Fe^{2+} was rapid with Tf-1, Tf-2, SM-3 and SM-4 with complete oxidation of Fe^{2+} in 25 hours, but SM-1, SM-2, SM-5 and SM-8 were slow growers.

CuSO_4 at 10 mM had no effect, but at 30-100 mM inhibited the growth of all strains by increasing the length of lag period, after which they all grew although more slowly.

All the strains tested grew at 100 or 150 mM ZnSO_4 with only minor inhibition.

The Fe^{2+} -oxidizing activity of all strains was inhibited by Fe^{3+} , Cu^{2+} and Zn^{2+} , generally competitively with respect to Fe^{2+} concentration.

In conclusion the fresh isolates were generally more tolerant of metals when grown on sulfur than laboratory strains which were maintained in laboratory media for a long period of time.

SESSION D'AFFICHAGE

RÉSUMÉ

Nous avons cultivé trois souches Thiobacillus thiooxidans (Tt = ATCC 8085, SM-6 et SM-7) et sept souches Thiobacillus ferrooxidans (Tf-1 = ATCC 13661, Tf-2 = ATCC 19859, SM-1, SM-3, SM-4, SM-5 et SM-8) sur du soufre élémentaire et huit souches T. ferrooxidans (Tf-1, Tf-2, SM-1, SM-2, SM-3, SM-4, SM-5 et SM-8) sur de fer ferreux comme source unique d'énergie. La tolérance en Cu et en Zn de ces souches a été étudiée aussi bien durant la croissance qu'avec des cellules de suspension au repos.

Au cours de la croissance sur soufre, SM-4, Tt, SM-3, SM-6 et SM-7 avaient une tolérance de 50 mM CuSO_4 ; SM-7, SM-6 et SM-3 toléraient 200 mM ZnSO_4 ; toutes les souches avaient une tolérance de 200 mM FeSO_4 . L'activité oxydante du soufre de SM-3, SM-4, SM-6 et SM-7 résistait à l'inhibition de 167 mM CuSO_4 et de 417 mM ZnSO_4 .

La croissance sur Fe^{2+} était rapide avec Tf-1, Tf-2, SM-3 et SM-4 avec oxydation complète de Fe^{2+} en 25 heures, mais SM-1, SM-2, SM-5 et SM-8 ne poussaient pas très rapidement.

A 10 mM CuSO_4 n'a aucun effet, mais à 30-100 mM il inhibe la croissance de toutes les souches en augmentant les temps de latence, après quoi elles croissent toutes, bien que plus lentement.

Toutes les souches qui ont été testées ont poussé à 100 ou 150 mM ZnSO_4 avec une seule inhibition mineure.

L'oxydation Fe^{2+} de toutes les souches a été inhibée par Fe^{3+} , Cu^{2+} et Zn^{2+} , généralement en compétition en ce qui concerne la concentration en Fe^{2+} .

En conclusion les souches nouvellement isolées étaient d'une façon générale plus tolérants des métaux lorsqu'ils sont cultivés sur du soufre que les souches de laboratoires qui ont été gardées dans un laboratoire média pendant longtemps.

DETERMINATION OF ACTIVITY PARAMETERS IN THIOBACILLUS FERROOXIDANS STRAINS AS CRITERIA FOR MINERAL LEACHING EFFICIENCY. I. GROWTH CHARACTERISTICS AND EFFECT OF METALS ON GROWTH AND ON SULFUR OF FERROUS IRON OXIDATION

MATERIALS AND METHODS

Thiobacillus thiooxidans ATCC 8085 (Tt), Thiobacillus ferrooxidans ATCC 19859 (Tf-2) were the laboratory control strains used. SM-1, SM-2, SM-3, SM-4, SM-5, SM-6, SM-7 and SM-8 were isolated from water samples from various seepage and drainage locations at the Flin Flon Mine of the Hudson Bay Mining and Smelting Co., Ltd., Manitoba. Tt, SM-6 and SM-7 grew only on sulfur as the energy source (T. thiooxidans) and was maintained in the Starkey's No. 1 medium (1). Tf-1, Tf-2, SM-1 to 5 and SM-8 grew on Fe^{2+} as the sole energy (T. ferrooxidans) and were maintained in a modified 9K (M9K) medium (2). T. ferrooxidans strains adapted to sulfur were maintained also in the Starkey's No.1 medium. All stock cultures were kept at 4°C.

Media and growth of bacteria on sulfur

The medium used for the growth on elemental sulfur was the Starkey's No. 1 medium (1): 0.3 g $(\text{NH}_4)_2\text{SO}_4$, 3.5 g KH_2PO_4 , 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.25 g CaCl_2 , and 18 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ per L of distilled water; adjusted to pH 2.3 with H_2SO_4 . Powdered precipitated sulfur (BDH Chemicals) (50 g/L) was spread evenly on the surface after inoculation (2.5% v/v) as described (3). Normally a culture was grown in one litre volume in a 2.8 L Fernbach flask at 25°C without agitation, but for some experiments it was grown in 200 mL volume in a 500 mL Erlenmeyer flask or at 28°C.

Cells were collected after 7 days by removing the remaining sulfur by filtration through a Whatman No. 1 filter paper under suction, by centrifuging the filtrate and washing the cells once in the Starkey's medium. The washed cells (20-50 mg wet cells/mL in the Starkey's medium) were used within two days for the determination of the sulfur-oxidizing activity.

For the study of heavy metal effect various concentrations of heavy metal solutions were adjusted to pH 2.3 with H_2SO_4 or NaOH and after sterilization added to the cooled sterile medium to avoid the precipitation with phosphate.

Growth rate on sulfur

Ten mL samples were removed periodically from cultures and bacterial cells were separated from elemental sulfur by filtration through a Whatman No.1 filter and were harvested by centrifugation. The cells were washed three times with the Starkey's No. 1 medium (pH 2.3) and then were boiled for 15 mins. in 0.1 N NaOH for the determination of total protein concentrations by the methods of Lowry *et al* (4) using crystalline bovine serum albumin as a standard. Growth curves were plotted on a semi-log scale to obtain the generation time.

Determination of sulfur-oxidizing activity

The rate of sulfur oxidation by various strains was determined by measuring the rate of oxygen disappearance in an Oxygraph with a Clark electrode at 25°C. The standard reaction mixture contained 50 μ L of a bacterial cell suspension (50 mg wet cells/mL), 0.3 mL sulfur (320 mg precipitated sulfur, low Fe, BDH/mL 500 ppm Tween 80) and Starkey's medium No. 1 (pH 2.3) in a total volume of 1.2 mL. The sulfur-oxidizing activity was expressed as nmol O_2 /min. The effect of heavy metals on the activity was studied by including in the reaction mixture 0.1 or 0.2 mL of 1 M $CuSO_4 \cdot 5H_2O$ or 2.5 M $ZnSO_4 \cdot 7H_2O$ at pH 2.3.

Media and growth of bacteria on iron

The medium used for the growth on ferrous iron was a modified 9K (M9K) medium (2): 0.4 g $(NH_4)_2SO_4$, 0.1 g K_2HPO_4 , 0.4 g $MgSO_4 \cdot 7H_2O$, and 33.3 g $FeSO_4 \cdot 7H_2O$ per litre and adjusted to pH 2.3 with H_2SO_4 . The ferrous sulfate solution was filter sterilized separately. Normally a culture was grown in 100 mL volume (10% inoculum) in a 250 mL Erlenmeyer flask at 25°C on a rotary shaker at 150 rpm, but for the purpose of obtaining cells for activity determination larger volumes and larger flasks were used. Cells were collected by centrifugation (12,000 x g for 10 min) after removal of ferric iron by decantation or low speed centrifugation (100 x g for 10 min).

Growth rate on iron

Stock cultures of various strains kept at 4°C were subcultured three times (each subculture at 75-80% consumption of ferrous iron) before the growth curve study of actively growing cultures was carried out.

Cell growth was followed by the determination of Fe^{3+} by the yellow color (5) in a Klett-Summerson Photoelectric Colorimeter with a blue (No. 42) filter after 0.5 mL culture sample was mixed with 4.5 mL 1 N HCl. Cell growth was also followed by the protein determination (4) to confirm that the amount of Fe^{3+} ion produced was proportional to the amount of protein in the culture during the growth. The protein determination was more difficult and was affected by the presence of ferric iron towards the end of growth phase. Therefore the simple Fe^{3+} determination was used throughout the growth study, except when cells were collected the protein concentration of cell suspensions was determined. The growth curves were plotted on a semi-log scale to obtain the generation time.

Determination of Fe^{2+} -oxidizing activity

The rate of ferrous iron oxidation by various strains was determined by measuring the rate of oxygen consumption in an Oxygraph at 25°C. The standard reaction mixture contained 25 μL of a bacterial suspension (50 mg wet cells/mL), microliter volumes of 0.1 M $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (pH 2.0) and M9K medium (pH 2.3) in a total volume of 1.2 mL. The Fe^{2+} -oxidizing activity was expressed as nmol O_2 /min. Routine assays were carried out with 10 or 50 μL of 0.1 M FeSO_4 , but for the characterization of each strain varied volumes of the FeSO_4 solution were used to obtain the maximal velocity, V_{max} , i.e., the activity at the saturating Fe^{2+} concentration, by the double reciprocal plots of activity versus the Fe^{2+} concentration.

The inhibitory effect of heavy metals on Fe^{2+} iron oxidation was studied by including in the reaction mixture microliter quantities of 1 M $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (pH 2.3), 2.5 M $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (pH 2.3) or 1 M $\text{Fe}_2(\text{SO}_4)_3$ in 0.1 M $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (pH 1.0) and measuring the rate of oxygen consumption at varying FeSO_4 concentrations. In

the face of Fe^{3+} inhibition the amount of Fe^{2+} added in the inhibitor solution had to be considered. The reaction was started with the addition of bacteria. The double reciprocal plots of activity versus Fe^{2+} concentrations at different heavy metal concentrations were analyzed for the type of inhibition, apparent K_m values for Fe^{2+} , and K_i values.

RESULTS

I. SULFUR-GROWN CELLS

Adaptation to sulfur

Tt, SM-6 and SM-7 were maintained on sulfur i.e. T. thiooxidans) and required no adaptation, but other strains were maintained on ferrous iron (i.e. T. ferrooxidans) and required adaptation periods before they grew well enough for study.

Tf-1, Tf-2, SM-3 and SM-4 adapted rapidly when transferred from the iron culture to the sulfur medium and reached the level of rapid growth equivalent to T. thiooxidans within one month (after 3-4 subcultures).

SM-1, SM-5 and SM-8 were more difficult to adapt. Initially it took them two to three weeks to reach the cell densities of one week old cultures of fully sulfur-adapted cells. Full adaptation to sulfur required 2 to 4 months of repeated transfer (3 to 4 subcultures for SM-1 and SM-3 and 6 to 7 subcultures for SM-5).

SM-2 never grew in the sulfur medium and therefore was considered incapable of using sulfur as energy source.

Characteristics of sulfur-grown cells

All strains, some after adaptation, grew rapidly on sulfur following a lag period of two to three days with a relatively uniform generation time of 7.8 to 9.5 hours and reached a cell protein concentration of 32 to 67 mg/L in 7 days at 25°C (Table 1, Figs. 1-3). As shown in Table 1 the cells collected by centrifugation had different colors characteristic of strains. The specific activity for sulfur oxidation varied four fold among different strains. Tt, Tf-1, Tf-2, SM-1, SM-6 and SM-7 cells had higher specific activities than SM-3, SM-4, SM-5 and SM-8 cells.

Effect of Cu and Zn on growth

Various strains adapted to sulfur were now grown on sulfur in the presence and absence of various heavy metals and the growth (mg protein/L) was followed for 7 days at 25°C. The effect of Cu^{2+} , Zn^{2+} and Fe^{2+} on the growth on sulfur is shown in Tables 2-4.

CuSO_4 had a clear-cut effect as shown in Table 2 in

distinguishing Cu-tolerant strains from Cu-sensitive strains, SM-4 being the most tolerant growing to 73% of control in the presence of 50 mM CuSO_4 (3.2 g Cu/L) and SM-8 the least tolerant growing only to 10% of control in 10 mM CuSO_4 (0.6 g Cu/L). Interestingly only the Cu-tolerant strains produced black cupric sulfide and H_2S odour, probably relating to their detoxification mechanism of Cu. Some CuS was inseparably bound to SM-6 cells.

Growth inhibition by ZnSO_4 required higher concentrations than CuSO_4 , but various strains were again placed in two groups, Zn-tolerant strains and Zn-sensitive strains. The former strains showed a graduation of tolerance, SM-7 and SM-6 being the most tolerant (52 and 34% growth of control at 200 mM ZnSO_4 or 13 g Zn/L) and SM-8 the least tolerant (Table 3). Both SM-1 and SM-5 were extremely sensitive reaching only 2-3% growth of control at 50 mM ZnSO_4 (3.2 g Zn). Thus SM-1 and SM-5 were very sensitive both to Cu and Zn during growth on sulfur with SM-8 being similar.

Table 4 indicates that all the strains studied were relatively tolerant of FeSO_4 during growth on sulfur at concentrations normally used for growth on ferrous iron (33.3 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}/\text{L} = 120 \text{ mM}$).

Growth curves for Tt, SM-6 and SM-7 with and without Cu or Zn are shown in Figs. 1 to 3. The general effects of Cu, Zn and Fe were to prolong the lag periods, to increase the generation time and to decrease the growth yields.

Effect of Cu and Zn on the sulfur-oxidizing activity

Various strains were grown on sulfur with and without 10 mM CuSO_4 or 50 mM ZnSO_4 and cells were harvested after 7 days for the cell yield and sulfur-oxidizing activity determinations with and without 167 mM CuSO_4 or 417 mM ZnSO_4 .

Table 5 shows the results of cells grown with and without Cu. The sulfur-oxidizing activity of control cells was affected by Cu and Zn differently among strains. SM-3, SM-4, SM-6, SM-7 and SM-8 were relatively more resistant to Cu and Zn inhibition of the activity (80-100% of control activity) than Tt, Tf-1, Tf-2, SM-1 and SM-5. SM-1 and SM-5 were particularly sensitive

to these metals both on the activity and growth (Tables 2 and 3). SM-3, SM-4, SM-6 and SM-7 were tolerant of these metals both on the activity and growth. SM-8, which was sensitive to the metals on growth, particularly to Cu, was not affected much on its sulfur-oxidizing activity.

Growth in the presence of 10 mM CuSO_4 increased the Cu resistance of cells on the sulfur-oxidizing activity in all the isolates tested (SM-8 did not grow). The Zn resistance generally decreased by the growth with Cu except in SM-5 and SM 6. Laboratory strains, Tt and Tf-1, became more sensitive to both Cu and Zn.

It should be mentioned that the effect of ZnSO_4 on the sulfur-oxidizing activity not reported in the table is that the lag period is increased extensively before a linear oxygen consumption is observed.

Table 6 shows the results with cells grown on sulfur with and without Zn, but this time at 28°C instead of 25°C because of an incubator problem, i.e., the sulfur-growing cultures inhibiting the growth of iron-growing cultures. Growth yield was generally higher at 28°C.

The sulfur-oxidizing activities of control cells grown at 28°C were considerably more sensitive to Cu and Zn than those cells grown at 25°C with the exception of Tf-2, SM-1 and SM-5 to Cu, as shown in Table 6. All strains became more sensitive to Zn, some extremely sensitive (e.g. SM-4). SM-3, SM-4, SM-6, SM-7 and SM-8, considered as resistant to the metal effect at 25°C, are now indistinguishable from other sensitive strains in their sensitivity to the metals on their sulfur-oxidizing activities.

Growth in the presence of 50 mM ZnSO_4 increased the Cu and Zn resistance of cells on the sulfur-oxidizing activity in all isolates, but decreased the resistance in some laboratory strains, Tt (Zn), Tf-1 (Cu) and Tf-2 (Zn).

II. IRON GROWN-CELLS

General growth characteristics

An initial growth study of various strains was carried out

both in the M9K medium and in the 9K medium (6) at pH 2.3, the latter having higher concentrations of salts than the former. The $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ concentration was set at 33.3 g/L (0.119 M) in both media.

As shown in Table 7 Tf-1, Tf-2, SM-3 and SM-4 all grew rapidly after a very short lag (2-3 hours) with a generation time of 5-7.5 hours and oxidized almost all the Fe^{2+} iron to the Fe^{3+} form (0.11 M Fe^{3+}) within 25 hours of shaking at 25°C, both in M9K and 9K media. Sometimes, particularly in the 9K medium growth curves showed biphasic lines, the first rapid growth phase followed by the slower phase leading to the depletion of Fe^{2+} .

SM-1, SM-2, SM-5 and SM-8 were slower due to extended lag periods or longer generation time or both. The 9K medium particularly extended the lag periods of SM-2 and SM-5. Growth time for complete Fe^{2+} oxidation varied from 25 hours in the fast growth group to 60 to over 100 hours in the slow growth group.

Growth yield either in mg protein or mg wet cells fluctuated considerably among strains (Table 7). Because of difficulty in removing ferric iron precipitate completely from cells the mg protein values are considered more reliable. Both Tf-1 and Tf-2 had high growth yields, but surprisingly SM-5, one of the slow growers, showed the highest growth yield. SM-2 had the lowest growth yield (2 mg protein/L) and the lowest iron-oxidizing activity per mg protein making it the strain with the lowest iron-oxidizing activity recoverable from 1 L of culture. Both growth yields and activities for the same strains fluctuated to same extent among experiments.

Effect of CuSO_4 on the growth

CuSO_4 at increasing concentrations (1-100 mM) increased both the length of lag period and the generation time as shown in Table 8 Figs. 4 and 5. Often in the presence of Cu there was an initial minor growth, normally corresponding to one cell division (Figs. 4 and 5), followed by an extended lag period before a major growth phase. In Tf-1 with 100 mM Cu the major growth phase was followed by a slower growth phase leading to the complete oxidation of Fe^{2+} to Fe^{3+} (Fig. 4).

In all strains the growth was little influenced by CuSO_4 up to 10 mM. CuSO_4 at 30 mM had little effect on Tf-2, SM-1 and SM-5, but increased the lag period considerably of Tf-1, SM-3, SM-4, and SM-8. CuSO_4 at 50 mM extended the lag period considerably of Tf-2 in addition to Tf-1, SM-3, SM-4 and SM-8, but affected SM-1 and SM-5 much less. CuSO_4 at 100 mM extended the lag period of all strains to 150 hours or longer except SM-1 and the generation time to two times (Tf-1, SM-1, SM-5, SM-8), four times (Tf-2) eight times (SM-4) and twelve times (SM-3). SM-2, a slow grower, grew more slowly than in experiments in Table 7, but the growth was little affected by Cu even at 100 mM. Three slow-growers (SM-1, SM-2 and SM-5) are considered more tolerant of Cu than other strains in their growth.

Effect of ZnSO_4 on the growth

The effect of ZnSO_4 on the growth of various strains is shown in Table 9. SM-1 and SM-2 were not included due to difficulty in their growth. The strains tested were all remarkably tolerant of Zn during growth on ferrous iron. At a concentration of 100 mM Zn had little effect on the growth of SM-4 and SM-8 and increased the lag period of SM-5 two times and both the lag period and generation time of SM-3 three times. Even at 150 mM Zn had little effect on Tf-1 and SM-4, only doubled the lag period of Tf-2 and the lag period and generation time of SM-8, and quadrupled the lag period and generation time of SM-3. Only SM-5 did not grow after 140 hours, although it grew at 100 mM Zn with the same generation time as the control after a lag period only twice that of control.

Effect of Fe^{2+} concentration on Fe^{2+} -oxidizing activity

The concentration of Fe^{2+} required for the half maximal velocity ($1/2 V_{\text{max}}$) was obtained as K_m by double reciprocal plots of Fe^{2+} -oxidizing activity versus Fe^{2+} concentrations and extending the line to the X-axis (Figs. 6,7). The K_m values for Fe^{2+} were very similar among various strains (Table 10) and were around 0.3-0.5 mM, except with Tf-1 and Tf-2, the two lab strains, and SM-2, the values were close to 1 mM. Sometimes biphasic plots were observed with higher Fe^{2+} concentrations

(>1 mM) activating the Fe^{2+} oxidation with higher K_m values (Fig. 7).

Effect of Fe^{3+} , Cu^{2+} , and Zn^{2+} on Fe^{2+} -oxidizing activity

Fe^{3+} inhibited the Fe^{2+} -oxidizing activity of all strains competitively with respect to the Fe^{2+} concentration (Fig. 6 and Table 10) in agreement with Kelly & Jones (7). K_i values for Fe^{3+} were relatively constant among strains, around 2 mM Fe^{3+} , except with SM-3 where the slope replot was biphasic with two K_i values. In all the strains therefore increasing Fe^{3+} concentrations increased the apparent K_m values for Fe^{2+} (i.e. the concentration required for half the maximal velocity).

The inhibition by Cu^{2+} was complex (Fig. 7 and Table 10). At high Fe^{2+} concentrations, Cu^{2+} inhibited the Fe^{2+} oxidation competitively, changing only the slope of the double reciprocal plots, except with SM-5. In the presence of Cu^{2+} the double reciprocal plots of most strains became distinctly biphasic at low Fe^{2+} concentrations with corresponding intercept changes. With Tf-1 at low Fe^{2+} the lines with Cu^{2+} became parallel to the control line without Cu^{2+} , thus changing only the intercept (uncompetitive inhibitions). With SM-5 the plots were still biphasic, but both intercept and slope changed at either high Fe^{2+} or low Fe^{2+} (noncompetitive). K_i values in Table 10 represented the measure of effect on the slope (Cu^{2+} concentration required for doubling of the slope) at high Fe^{2+} (K_i slope) and effect on the intercept (Cu^{2+} concentration required for doubling of the intercept) at low Fe^{2+} . The K_i values for Cu^{2+} in Table 10 varied from 30 mM to 155 mM. At high Fe^{2+} the K_i was fairly constant among isolates (30-70 mM), except SM-2. At low Fe^{2+} SM-3 and SM-4 showed higher K_i values (150 and 155 mM Cu^{2+}) compared to others.

The inhibition by Zn^{2+} was similar to Cu^{2+} . All strains were competitively inhibited by Zn^{2+} at high Fe^{2+} . At low Fe^{2+} the inhibition plots of some strains became biphasic with intercept changes.

Comparison of metal effects on growth and on activity

The K_i values of various strains did not relate easily to

their response to Cu and Zn during growth (Tables 8 and 9). Under the growth conditions reported the K_i values for high Fe^{2+} concentrations are considered to be more relevant. During the growth on Fe^{2+} SM-1, SM-2 and SM-5 were found to be most tolerant of Cu but the Fe^{2+} -oxidizing activities of SM-1 and SM-5 were sensitive to Cu^{2+} and Zn^{2+} reported in Table 10 are too large to have a major effect on binding of Fe^{2+} under the growth experiments. For example, SM-4 has a K_m value for Fe^{2+} of 0.46 mM and a K_i value for Cu^{2+} of 30 mM. Since the K_m value increases by a factor of $[I]/K_i$ in competitive inhibition by an inhibitor I at a concentration [I], the highest Cu concentration used for growth experiment of 100 mM, the apparent K_m value will go up to 2 mM Fe^{2+} ($0.46 \text{ mM} + 0.46 \text{ mM} \times 100 \text{ mM}/30 \text{ mM}$). In the growth medium containing 33.3 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}/\text{L}$ (120 mM) the Fe^{2+} oxidizing system of bacteria will still be 98% saturated with Fe^{2+} , since $[S]/(K_m + [S])$ will be $120 \text{ mM}/2 \text{ mM} + 120 \text{ mM}$, where [S] is the concentration of substrate, Fe^{2+} . The system therefore will be oxidizing Fe^{2+} almost without inhibition.

The metal tolerance of T. ferrooxidans during growth must involve not only the inhibition by metals of the Fe^{2+} -oxidizing activity, but also the inhibition of other biochemical reactions essential for growth which are probably more sensitive to metal inhibition if exposed. The extended lag period is very likely the period to detoxify or exclude the metals from the inside of cells so that the necessary reactions for cell division can take place. The complex nature of Cu and Zn inhibition particularly at low Fe^{2+} concentrations (Table 10) may have a significant implication on the metal tolerance in bacterial leaching where the concentration of Fe^{2+} will be much lower than our growth conditions (120 mM Fe^{2+}).

Continuing work

Adaptation of sulfur-growing the iron-growing strains to Cu and Zn is continuing. Our preliminary results indicate that the lag periods can be shortened during adaptation. These adapted cells should provide us more precise information on the metal tolerance of various strains.

In conclusion, we have shown that fresh isolates from the mine sites are generally more tolerant of metals when grown on sulfur than laboratory strains which were maintained in laboratory media for a long period of time.

ACKNOWLEDGEMENTS

The authors wish to thank CANMET for a contract (DSS Contract 234409126) in support of this work.

REFERENCES

1. Starkey, R.L. "Concerning the physiology of Thiobacillus thiooxidans, an autotrophic bacterium oxidizing sulfur under acid conditins"; J. Bacteriol. 10: 135-163; 1925.
2. McCready, R.G.L., Wadden, D., and Marchbank, A. "Nutrient requirements for the in-place leaching of uranium by Thiobacillus ferrooxidans"; Hydrometallurgy 17: 61-71; 1986.
3. Suzuki, I. "Oxidation of elemental sulfur by an enzyme system of Thiobacillus thiooxidans"; Biochim. Biophys. Acta 104: 359-371; 1965.
4. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. "Protein measurement with the Folin reagent"; J. Biol. Chem. 193: 265-275; 1951.
5. Schnaitman, C.A., Korczynski, M.S., and Lungren, D.G. "Kinetic studies of iron oxidation by whole cells of Ferrobacillus ferrooxidans"; J. Bacteriol. 99: 552-557; 1969.
6. Silverman, M.P., and Lundgren, D.G. "Studies on the chemoautotrophic iron bacterium Ferrobacillus ferrooxidans. II. Manometric studies"; J. Bacteriol. 78: 326-331; 1959.
7. Kelly, D.P., and Jones, C.A. "Factors affecting metabolism and ferrous iron oxidation in suspensions and batch cultures of Thiobacillus ferrooxidans: Relevance to ferric iron leach solution regeneration"; Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena; L.E. Murr, A.E. Torma, and J.A. Brierley, Editors; Academic Press; New York; pp. 19-44; 1978.

TABLES

TABLE 1 GROWTH CHARACTERISTICS, SULFUR-OXIDIZING ACTIVITY AND COLOR OF VARIOUS STRAINS GROWN AT 25°C ON SULFUR FOR 7 DAYS

Strains	Lag period days	Generation time hours	Growth yield/L		Activity nmol O ₂ /min per mg protein	Color of packed cells
			mg protein	mg wet cells		
Tt	3	8.0	51	400	220	milky white
Tf-1	2	7.8	45	250	187	bright yellow
Tf-2	2	8.7	41	250	224	bright yellow
SM-1	3	8.9	67	250	208	brownish grey
SM-3	3	9.5	32	100	51	bright yellow
SM-4	3	8.1	37	125	76	bright yellow
SM-5	2	7.8	47	250	89	milky white
SM-6	3	8.4	41	400	273	yellow
SM-7	2	8.9	59	300	196	brownish grey
SM-8	2	8.4	62	400	65	milky pale yellow

SM-2: No growth on sulfur.

TABLE 2 EFFECT OF CuSO_4 ON THE GROWTH OF VARIOUS STRAINS GROWN
AT 25°C ON SULFUR FOR 7 DAYS

Strains	Growth (% of control in mg protein/L		
	10 mM CuSO_4	30 mM CuSO_4	50 mM CuSO_4
Tt	97	22	15
Tf-1	62	0.5	0.5
Tf-2	81	0.5	0.5
SM-1	51	1.6	1.1
SM-3	56	80	9.3
SM-4	80	87	73
SM-5	41	2.8	1.8
SM-6	34	37	12.5
SM-7	82	51	10
SM-8	10	7.7	1.1

Cu - tolerant: SM-4 (most tolerant), Tt, SM-3, SM-6, SM-7
All produce black CuS , tightly bound to SM-6 cells.

Cu - sensitive strains: Tf-1, Tf-2, SM-1, SM-5, SM-8 (most sensitive).
None produces CuS .

TABLE 3 EFFECT OF ZnSO_4 ON THE GROWTH OF VARIOUS STRAINS GROWN AT 25°C ON SULFUR FOR 7 DAYS

Strains	Growth (% of control in mg protein/L)		
	50 mM ZnSO_4	100 mM ZnSO_4	200 mM ZnSO_4
Tt	32	28	1.2
Tf-1	48	15	4.6
Tf-2	28	5.0	2.5
SM-1	2.8	3.5	0.8
SM-3	92	15	13
SM-4	22	5.1	2.9
SM-5	2.4	2.8	1.0
SM-6	62	29	34
SM-7	99	69	52
SM-8	23	4.3	0.9

Zn-tolerant strains: SM-7 and SM-6 (most tolerant), SM-3, Tf-1, SM-4, Tf-2, Tt, SM-8 (least tolerant)

Zn-sensitive strains: SM-1, SM-5

TABLE 4 EFFECT OF FeSO_4 ON THE GROWTH OF VARIOUS STRAINS GROWN
AT 25°C ON SULFUR FOR 7 DAYS

Strains	Growth (% of control in mg protein/L)		
	50 mM FeSO_4	100 mM FeSO_4	200 mM FeSO_4
Tt	74	67	27
Tf-1	96	93	55
Tf-2	62	46	25
SM-1	83	52	26
SM-3	70	48	37
SM-4	74	44	33
SM-5	102	95	40
SM-6	72	35	25
SM-7	65	31	14
SM-8	119	68	21

All strains are Fe^{2+} tolerant.

TABLE 5 EFFECT OF CU AND ZN ON THE SULFUR-OXIDIZING ACTIVITY OF VARIOUS STRAINS GROWN AT 25°C ON SULFUR FOR 7 DAYS WITH AND WITHOUT 10 mM CuSO_4

Strains	Activity (% of control without Cu or Zn)			
	167 mM Cu		417 mM Zn	
	Cells (0 mM Cu)	Cells (10 mM Cu)	Cells (0 mM Cu)	Cells (10 mM Cu)
Tt	74	70	57	54
Tf-1	79	65	48	42
Tf-2	70	73	40	37
SM-1	53	77	43	32
SM-3	95	100	88	73
SM-4	88	95	90	67
SM-5	69	76	36	48
SM-6	97	110	77	95
SM-7	92	96	81	72
SM-8	84	-	92	-

All isolates tested become more Cu-resistant after growth with 10 mM Cu.

TABLE 6 EFFECT OF CU AND ZN ON THE SULFUR-OXIDIZING ACTIVITY OF VARIOUS STRAINS GROWN AT 28°C ON SULFUR FOR 7 DAYS WITH AND WITHOUT 50 mM ZNSO₄

Strains	Activity (% of control without Cu or Zn)			
	167 mM Cu		417 mM Zn	
	Cells (0 mM Zn)	Cells (10 mM Zn)	Cells (0 mM Zn)	Cells (10 mM Zn)
Tt	67	73	51	42
Tf-1	73	64	12	22
Tf-2	82	88	35	29
SM-1	56	85	15	19
SM-3	62	77	23	24
SM-4	72	78	9	21
SM-5	69	86	12	23
SM-6	58	86	36	43
SM-7	63	76	31	33
SM-8	60	74	13	30

All isolates tested become more Cu- and Zn-resistant after growth with 50 mM Zn.

TABLE 7 GROWTH CHARACTERISTICS AND IRON-OXIDIZING ACTIVITY OF VARIOUS STRAINS grown at 25°C ON FERROUS IRON

Strains	Growth medium	Lag period hours	Generation time hours	Growth time** hours	Growth yield/L mg protein	Growth yield/L mg wet cells	Activity (V_{max}) per mg protein nmol O_2 /min
Tf-1	M9K	2-3	6.8	25	8.8	83	1250
	9K	2-3	5.5(10)*	25			
Tf-2	M9K	2-3	6.8	25	15.4	80	1250
	9K	2-3	5.0(10)*	25			
SM-1	M9K	15	9.6(35)*	60	2.6	20	680
	9K	2-3	12.0(29)*	55			
SM-2	M9K	2-3	18.8	70	2.0	25	190
	9K	80	18.0	130			
SM-3	M9K	2-3	6.0(8.5)*	25	7.2	49	230
	9K	2-3	6.0(10)*	25			
SM-4	M9K	2-3	7.5	25	3.4	40	1740
	9K	2-3	7.0	25			
SM-5	M9K	20	21.0	130	49.1	332	800
	9K	225	24.4	275			
SM-8	M9K	10	45.6	140	5.0	28	690
	9K	10	30.4	100			

* Generation time during the second and slower growth phase before Fe^{2+} depletion.

** Incubation time at 25°C for complete oxidation of Fe^{2+} to Fe^{3+} .

Fast growers: Tf-1, Tf-2, SM-3, SM-4

Slow growers: SM-1, SM-2, SM-5, SM-8

TABLE 8 EFFECT OF CuSO_4 ON THE GROWTH OF VARIOUS STRAINS ON FERROUS IRON AT 25°C

Strains	lag period (LP) or generation time (Gt) in hours					
	CuSO_4 Concentrations					
	0 mM	1 mM	10 mM	30 mM	50 mM	100 mM
Tf-1 (Lp)	4	4	8	(12)* 94	(12)*156	(12)*244
(Gt)	7	7	10	(30)* 10	(30)* 11	(30)*16(110)**
Tf-2 (Lp)	2	2	2	3	(4)* 90	(4)*155
(Gt)	8	8	8	9	(19)* 11	(19)* 29
SM-1 (Lp)	12	12	12	17	30	88
(Gt)	13	13	13	13	22	32
SM-2 (Lp)	(2)*208		(2)*208		(2)*208	(2)*208
(Gt)	(60)* 45		(64)* 46		(64)* 45	(64)* 60
SM-3 (Lp)	2	2	2	(2)*134	(2)*220	(2)*300
(Gt)	6	6	10(15)**	(24)* 14	(24)* 28	(24)* 70
SM-4 (Lp)	4	4	4	(4)* 80	(4)*140	(4)*280
(Gt)	7	7	11	(15)* 22	(15)* 28	(15)* 57
SM-5 (Lp)	25	25	25	28	38	(50)*160
(Gt)	17(40)**	17(40)**	17(40)**	20(80)**	28(80)**	(107)*33
SM-8 (Lp)	6	6	6	36	64	158
(Gt)	17	17	17	23	23	36

* Lag period and generation time for the initial minor growth before an extended lag followed by the major growth.

** Generation time during the slower growth phase before Fe^{2+} depletion.

TABLE 9 EFFECT OF ZnSO_4 ON THE GROWTH OF VARIOUS STRAINS ON FERROUS
IRON AT 25°C

Strains	Lag period (Lp) or generation time (Gt) in hours					
	ZnSO_4 concentrations					
	0 mM	10 mM	50 mM	100 mM	150 mM	
Tf-1 (Lp)	2				2	
(Gt)	8				9	
Tf-2	(Lp)	18				32
	(Gt)	9				9
SM-3	(Lp)	2	3	3	6	8
	(Gt)	10	10	21	34	46
SM-4	(Lp)	2	2	3	3	4
	(Gt)	12	12	12	12	15
SM-5 (Lp)	38	68	95	80	<140	
	(Gt)	11	16	15	11	
SM-8 (Lp)	38		50	57	63	
	(Gt)	18		18	20	29

TABLE 10 EFFECT OF Fe^{2+} , Fe^{3+} , Cu^{2+} AND Zn^{2+} CONCENTRATIONS ON THE FERROUS IRON OXIDIZING ACTIVITY OF VARIOUS STRAINS

Strain	Km	Inhibition							
		Fe^{3+}		Cu^{2+}		Zn^{2+}			
		Type*	K_i (mM)	Type*		K_i (mM)	Type*		K_i (mM)
	mM Fe^{2+}			High Fe^{2+}	Low Fe^{2+}		High Fe^{2+}	Low Fe^{2+}	
Tf-1	1.2	C	1.5	C	UC	83(s)** 50(i)**	C	UC	75(s) 80(i)
Tf-2	0.95	C	1.8	C	NC	130(s) 90(i)	C		142(s)
SM-1	0.35	C	1.9	C		54(s)	C		77(s)
SM-2	1.0	C	2.0	C		130(s)	C		300(s)
SM-3	0.20 0.59	C	1.2 10.0	C	NC	70(s) 150(i)	C	NC	180(s) 180(i)
SM-4	0.46	C	2.0	C	NC	30(s) 155(i)	C	NC	46(s) 215(i)
SM-5	0.43	C	1.5	NC	NC	50(s) 75(i)	C	NC	55(s) 160(i)
SM-8	0.33	C	2.0	C	NC	68(s) 60(i)	C	NC	116(s) 120(i)

*Type C: Competitive; no intercept change, only slope change.

UC: Uncompetitive; no slope change, only intercept change.

NC: Noncompetitive; both intercept and slope change.

High Fe^{2+} , Low Fe^{2+} : Biphasic pattern with one type of inhibition at high Fe^{2+} concentration and another type at low Fe^{2+} concentrations.

**s = slope, i = intercept.

FIGURES

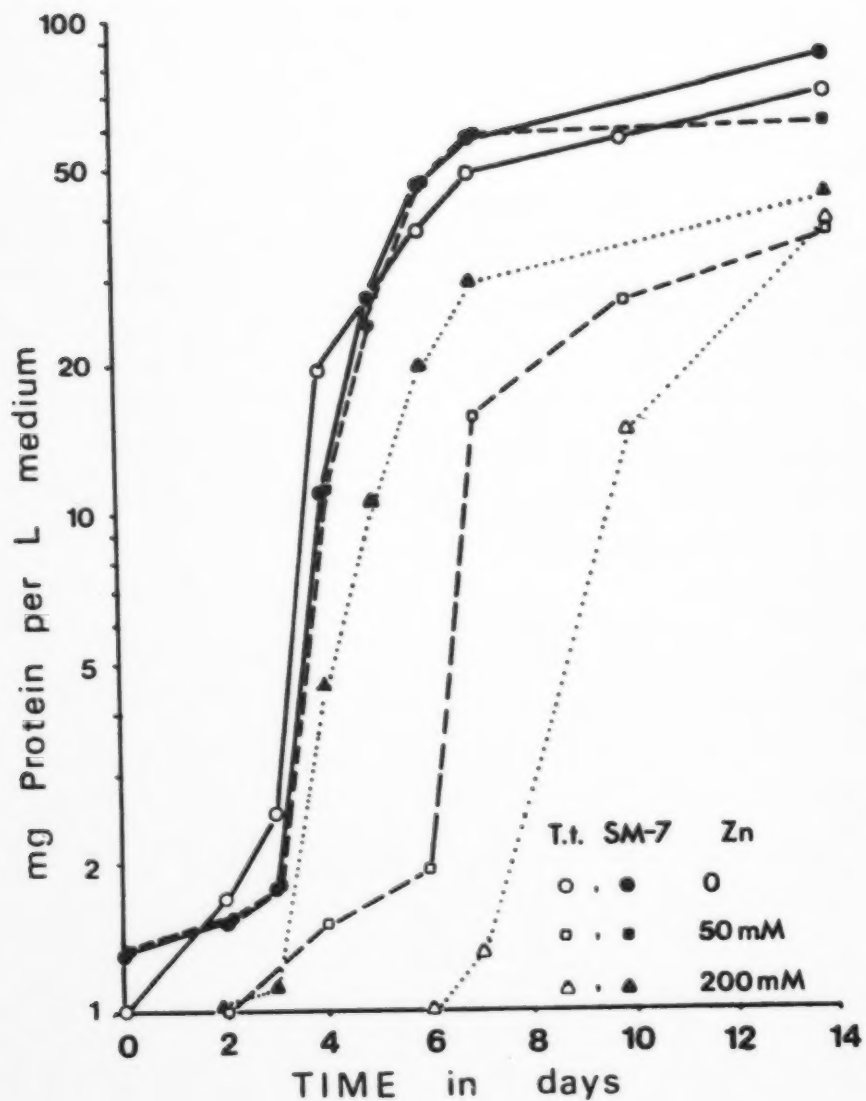


Fig. 1. Growth of *T. thiooxidans* ATCC 8085 (Tt) on sulfur with and without Cu^{2+} .

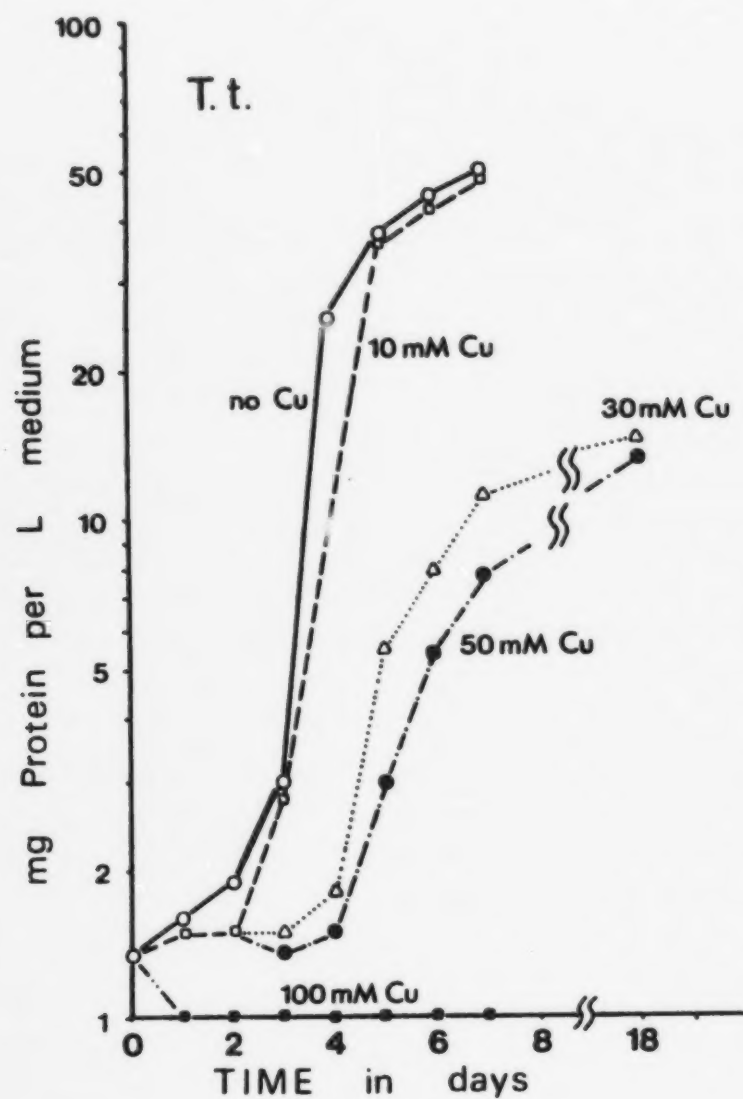


Fig. 2. Growth of SM-7 on sulfur with and without Cu^{2+} .

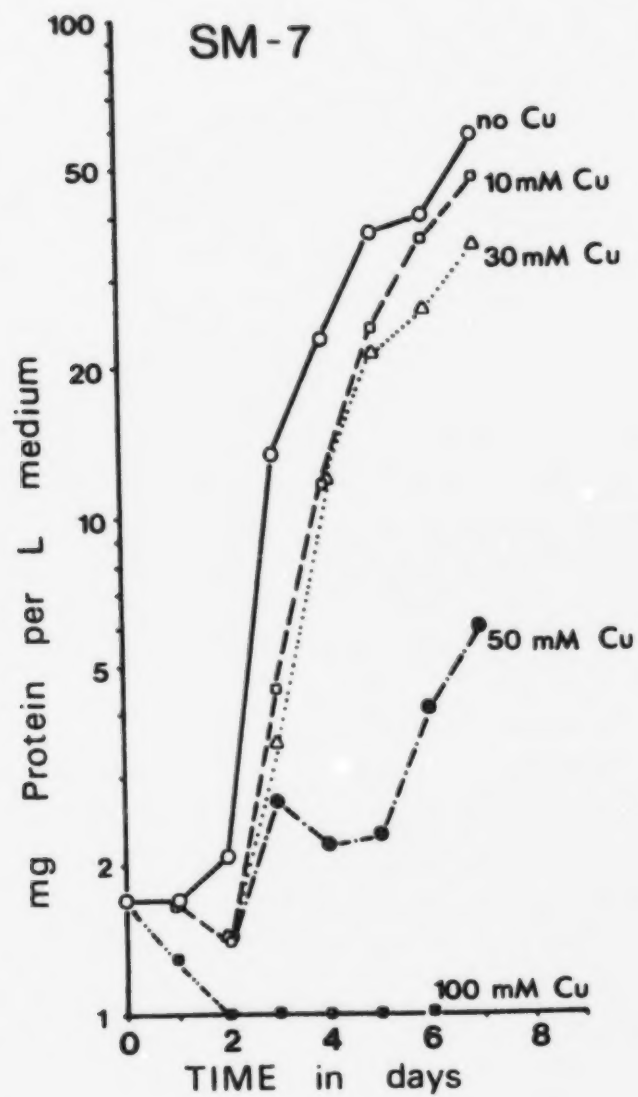


Fig. 3. Growth of Tt and SM-7 on sulfur with and without Zn^{2+} .

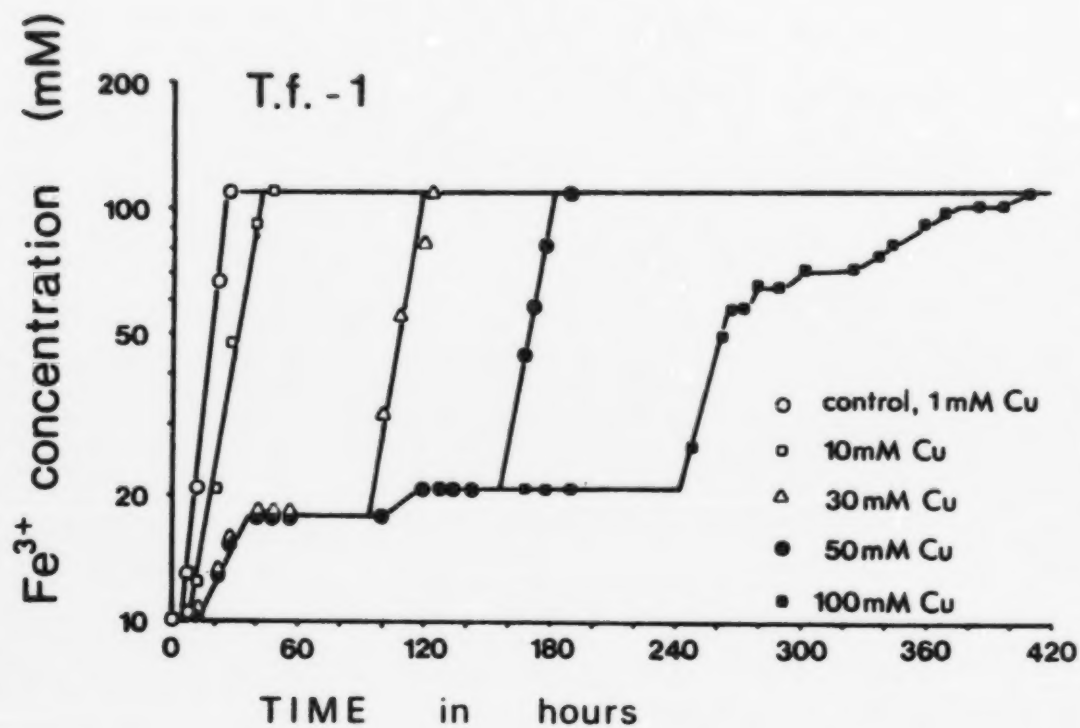


Fig. 4. Growth of *T. ferrooxidans* ATCC 13661 (Tf-1) on Fe²⁺ with and without Cu²⁺.

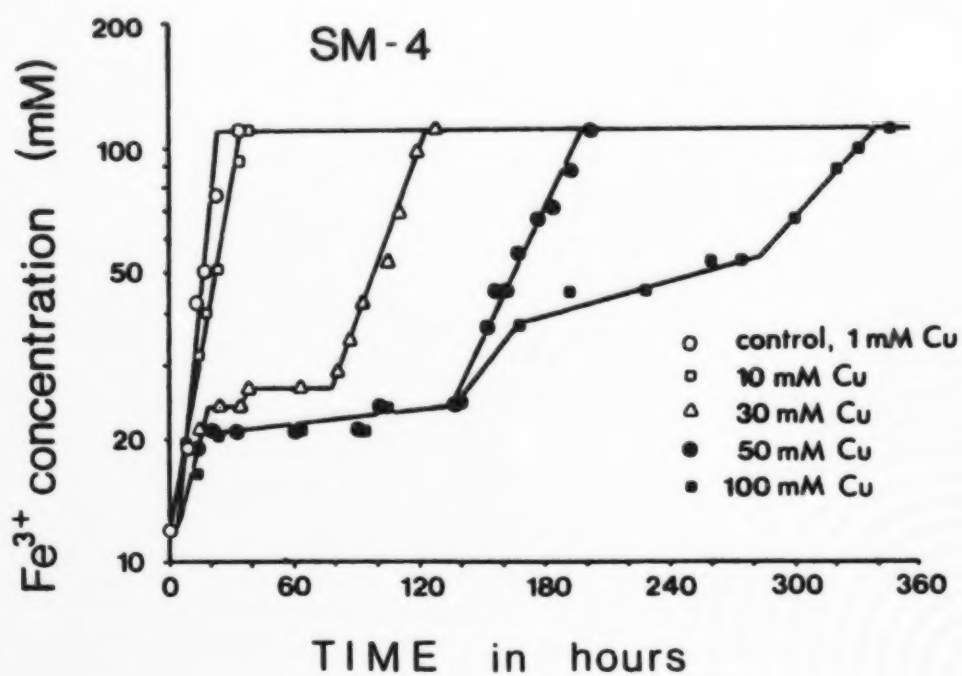


Fig. 5. Growth of SM-4 on Fe²⁺ with and without Cu²⁺.

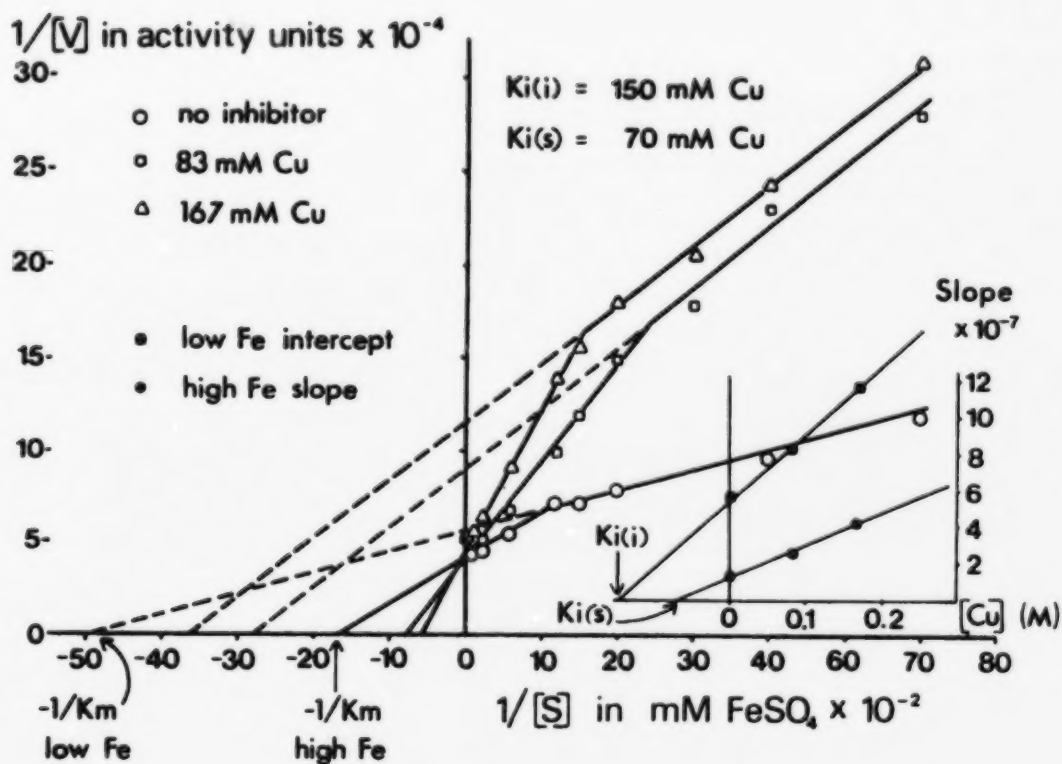


Fig. 6. Effect of Fe^{2+} concentration on the Fe^{2+} oxidizing activity of SM-3 and its inhibition by Cu^{2+} .

$1/V$ in activity units $\times 10^{-3}$

